Biochemistry

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Name: ______________________________

Subject: Biochemistry

Poonam Agrawal Mam

Date 18/2/18
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BIOCHEMISTRY

CARBOHYDRATE METABOLISM

Carbohydrate → all compounds → aldehyde or Keto derivative of polyhydroxy alcohol

Glycerol → Parental alcohol & gives carbohydrate in nature
[3–OH]

$$\begin{array}{c} CH_2-OH \\ | \\ CH-OH \\ | \\ CH_2-OH \end{array} \xrightarrow[\text{derivative}]{\text{Aldehyde}} \begin{array}{c} CHO \\ | \\ CH-OH \\ | \\ CH_2-OH \end{array}$$

[Glycerol] – D

[Glyceraldehyde] → D, L → Parent carbohydrate

1st carbohydrate derived in nature

Hydrolysis of compound

depending on No. of monosaccharide unit left after hydrolysis of compound – is basis of classification.

- Monosaccharide (Simple carbohydrate)
- Disaccharide (2 monosaccharide)
- Oligosaccharide (3–10 monosaccharide)
- Polysaccha (>10 Mon

Monosaccharide → Can't be hydrolysed further → Simplest monosaccharide → Glyceraldehyde

Monosaccharide → $C_n(H_2O)_n$

n → No. of C-atom

n → 3-9

n	Trivial Name	Aldose	Ketose
3	Trioses $C_3H_6O_3$	Glyceraldehyde	Dihydroxy acetone
4	Tetroses $C_4H_8O_4$	Erythrose	Erythrulose
5	Pentoses $C_5H_{10}O_5$	Ribose Xylose Arabinose Lyxose	Ribulose Xylulose x x
6	Hexose $C_6H_{12}O_6$	Glucose Galactose Mannose	Fructose
7	Heptose $C_7H_{14}O_7$	Glucoheptose	Sedoheptulose
8	Octose	x	x
9	Nonnose	Neuraminic acid [NANA] [N-acetyl Neuraminic acid] also known as "sialic acid". Found in "ganglioside".	

ISOMERS OF CARBOHYDRATE

Same formula ē different structures
↳ chemical / Empirical / Molecular

1. Aldose & Ketose isomerism [–CHO & –C=O]
2. Epimers
3. Pyranose & Furanose ring structures
4. α & β anomer
5. D & L type

EPIMERS → difference in structure against one** asymmetric C-atom

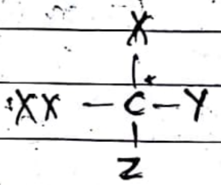
Glucose & Galactose → C_4
Glucose & Mannose → C_2

C_2 M → Mannose
C_3 A → Allose
C_4 G → Galactose
C_5 I → Idose

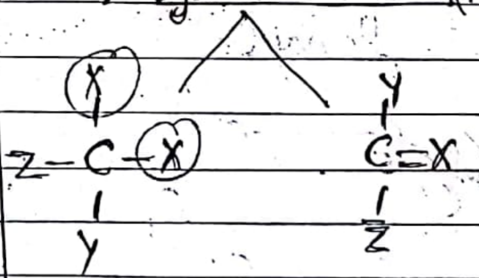
Galactose & Mannose ✗

Glucose →
- 2 Symmetric C-atom [C_1 & C_6]
- 4 asymmetric C-atom [C_2, C_3, C_4, C_5]

Asymmetric C-atom ↓ Chiral C-atom



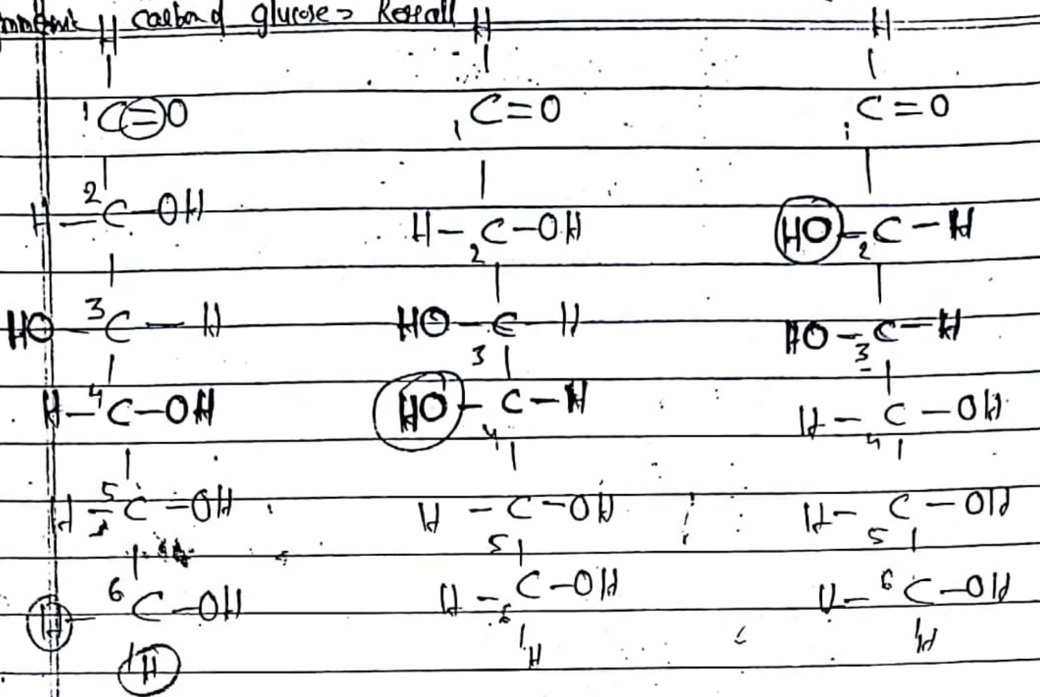
Symmetric C-atom / Achiral C-atom



Minm 2 valency ē same atom or 2 valencies utilize to add one atom

- Glucose also k/as "Dextrose".

* Symmetric carbon of glucose = 1, 6
Asymmetric carbon of glucose = Recall



Glucose	Galactose	Mannose

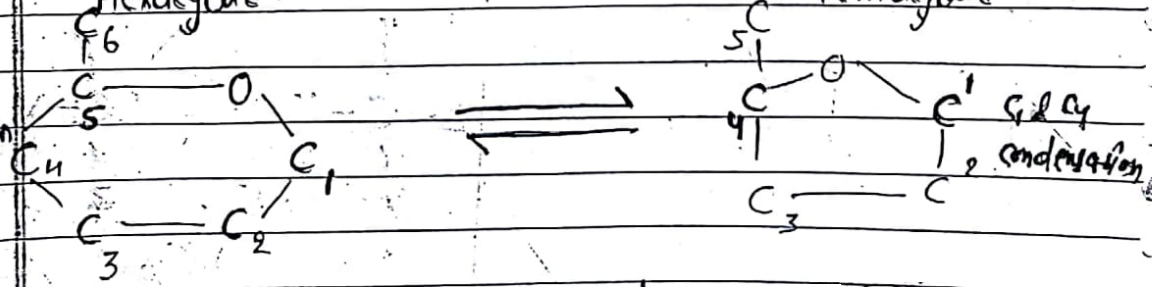
diff in 2 asymmetric C → Not epimers

Ribose – xylose } epimers
Ribulose – xylulose }

PYRANOSE & FURANOSE

PYRANOSE	FURANOSE
Heterocyclic (made of C & other atoms)	Heterocyclic
Hexacyclic	Pentacyclic

C_1 & C_5 condensation



- Glucose in aq. Media can form both ring, but initially glucose is in Pyranose form; later can convert to each other

100% Glucose converted in this form in aq. media

Teacher's Signature ....

Glucose – Precursor < Pyranose, α-glucose, β-glucose

* Pyranose & Furanose form seen in Minm 6 carbon-compound.

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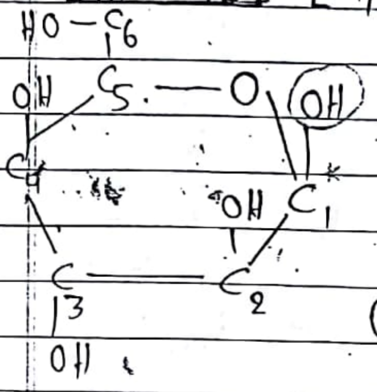
eqm is a kind of dynamic state (Rate of Forward Rxn equal to Rate of backward Rxn)

Q. Precursor form of glucose = Pyranose [also for Fructose]

At equilibrium predominent form = Pyranose [For glucose] (95%)

At equilibrium predominent form of fructose = Pyranose

[ANOMERS] [α/β]



$\boxed{C_1 - \text{anomeric carbon}}$

↓

Glucose, Galactose, Mannose

Beta

$\boxed{C_2 - \text{anomeric}}$ → For fructose

Anomeric C-atom

↓

Bears the reactive group & it is involved in Ring closure by condensation c Same hydroxyl group.

OR

carbon atom which was symmetric before & becomes asymmetric after the Ring closure

Trick: (AB)

If –OH is above the plane on Anomeric carbon

↓

Beta Anomer

al –OH is below the plane on Anomeric carbon

↓

Alpha anomer.

[MUTAROTATION] → Exclusive for glucose solution (Hexose solution)

$\boxed{\text{Glucose solution}}$ $\xrightarrow{\text{Fresh}}$ check optical activity ⟶ Rotation of optical light by $\boxed{+112°}$ → mean +112° on Rt. side

↓ MR

after 12 hrs

↳ optical activity ↓ to $\boxed{+52.5°}$

Teacher's Signature

Date
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glucose → always dextro-rotatory

Optical activity < $\alpha = +112^\circ$; $\beta = +19^\circ$

∴ in fresh solution predominent form ⇒ "α"
↓ Precursor of glucose

↓ Later

$\alpha \rightleftharpoons \beta$ (Interconversion of α into β is the reason behind MutaRotation)

at equilibrium ↓ ↓

$\boxed{1/3\alpha + 2/3\beta} = +52.5^\circ$ optical activity.
↓
Predominent at equilibrium

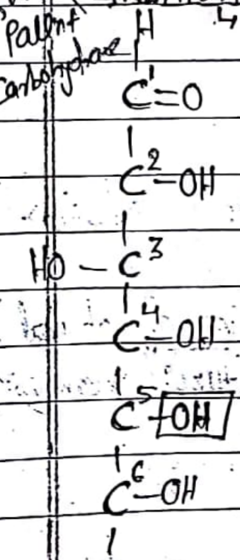
· Phenomenon of change (↓) in optical activity ē time in glucose solution → Mutarotation

$\boxed{\text{D \& L ISOMERS}}$ → Look for position of –OH on C_5 (Enantiomers) or Penultimate/Reference C-atom

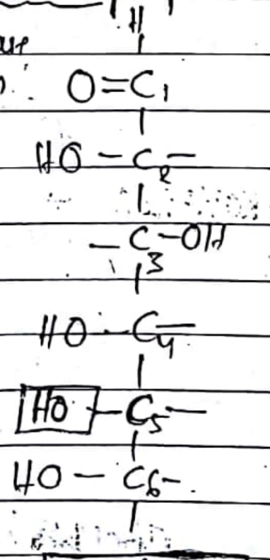
↳ Mirror image

glyceraldehyde (Parent Carbohydrate)

before Last carbon in compound



$\boxed{\text{D- glucose}}$



$\boxed{\text{L- glucose}}$

Teacher's Signature ....

- Pancreatic amylase → acts on internal α-1,4 glycosidic bond
- Lactulose → Not broken in GIT.

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D & L forms → K/as "Enantiomers" | d/l → optically active

Optically inactive — Mirror image of each other.

- Carbohydrate are predominent in D-form.
- AA predominent in L-form

Disaccharides	Units	Bonds
Sucrose (NR)	(α) Glu + (β) Fru	α-D-glucopyranosyl β-D-fructofuranoside
Trehalose (NR)	Glu + Glu	α 1,1 glycosidic
Lactose (R)	Glu (4) + Gal (1)	β-1,4 glycosidic bond [glu is β]
Lactulose (R)	Fru + Gal	β-1,4 glycosidic bond [Fru is β]
Maltose (R)	Glu + Glu	α-1,4 glycosidic
Isomaltose (R)	Glu + Glu	α-1,6 glycosidic

*NR → Non-Reducing
R → Reducing
↳ Reduce cupric [Cu^{+2} of $CuSO_4$] of Benedict's solⁿ → Semiquantitative assessment
Blue → Cuprous [Cu^{+}] → Brick Red

- Reducing Sugar in urine can be detected by "Benedict's solⁿ; Fehling Solⁿ; glucose-oxidase test".

Teacher's Signature

Monosaccharides → Reducing Sugar [free Reactive group]

* Criteria for Reducing tendency → Free aldehyde & free ketone group
↓
form Hemiacetal & Hemiketal

* Sucrose
- α-D-glucose (Pyranose ring)
- +
- β-D-fructose (Furanose Ring)

Reducing tendency of Lactose is due to "Glucose".

Reducing tendency of Lactulose is due to "Fructose".

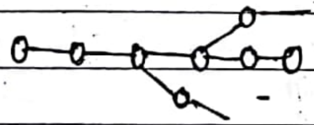
Oligosaccharide

OLIGOSACCHARIDES

3-10 monosaccharides

eg → Malto-triose [glucose - glucose - glucose]
Limit dextrin [8-10 glucose c̄ slight Branching]

end product of starch a/n digestion & glycogen in human.



→ present in cell membrane as a part of
Glycolipid
Glycoprotein.

Sometimes Antigenic (usually proteins are antigenic)

Play role in Receptor activity

Teacher's Signature ....

Oxidase & peroxidase → used for estimation of glucose

glucose + H_2O + O_2 $\xrightarrow{\text{oxidase}}$ H_2O_2 + Glyconic acid

KI ↓ Peroxidase

Brown Iodide

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Straight (Linear Amylose form)

POLYSACCHARIDE

HOMOPOLYSACCHARIDE

20% Amylose + 80% Amylopectin → Lesser branched than glycogen

Compound	Monomer	Bonds
Starch	glucose	α-1,4 , α-1,6
Glycogen (Branched)	glucose	α-1,4 , α-1,6
Cellulose (Linear)	glucose	β-1,4
Inulin (Linear)	Fructose	β-1,2
Dextran (Branched)	glucose	α-1,6 | 1,4 | 1,3
Chitin (Linear)	N-acetyl D-glucosamine (NAG)	β(1→4)

Cellulose → Animals digest it; we can't digest (we lack cellulase) — cellulose β-1,4

Inulin → used to assess the GFR.

Dextran → Plasma expander in hypovolemic shock

Chitin → Found in exoskeleton of crustacean (invertebrate) eg → Insects.

HETEROPOLYSACCHARIDE

↓

Mucopolysaccharides (GAG)

↓

glycosaminoglycans

glucose:

- oxidase → only –CHO oxidized to –COOH → gluconic acid (Aldonic Acid)
- Both –CHO & –OH is oxidized → glucosaccharic acid
- only terminal 1° OH is oxidized → glucuronic acid OR Uronic Acid

GAGs — 6 groups

1. Hyaluronic acid
2. Heparin [Most Negative]
3. Heparan Sulphate
4. Chondroitin Sulphate
5. Dermatan sulphate
6. Keratan Sulphate → Not Keratin, it is protein

Teacher's Signature ....

*** cellulose ⇒ β-(1,4) L glucose

$[O] + 4AAP \Rightarrow$ Quinoneimine complex (Pink)
(4-Amino Antipyrine)

* gluconic acid used in the glucose estimation by GOD-POD Method
(glucose oxidase, Peroxidase)

[Glucose $\xrightarrow{GOD}$ gluconic acid + H_2O_2] [$H_2O_2 \xrightarrow{POD} H_2O + [O]$]

- Mucopolysaccharides are made up of Amino Sugars & Uronic acids [UA - AA - UA - AA → Random Repeats]

QQ Linear compound

→ Highly Sulphated except → * Hyaluronic acid → No Sulphation

- Heparin → highly Sulphated.
- * Heparin > Hepa Sulphate (order of sulphation)
- Uronic acid → Oxidized form of Monosaccharides

↓

Last carbon [1° alcohol group]

↓

$CH_2-OH \rightarrow COOH$.

- Amino Sugar → -OH is Replaced by $-NH_2$ in Monosaccharides
- Kera-tan Sulphate → No uronic acid.

- Iduronic acid → 5' epimer of glucuronic acid.

Found in
- heparin
- heparin Sulphate
- Dermatan Sulphate

} prst in place of uronic Acid.

- Mucopolysaccharides are * polyanions [Negative charges]

↓

∴ MPs are hygroscopic (absorb H_2O)
∵ Polyanions

- MPs are important component of proteoglycans *

↓

(+) in Extracellular Matrix

∴ MPs are responsible for Turgidity of ECM

Teacher's Signature..................

- Amino Sugars are formed from "Fructose-6-P".
- Pyruvate Kinase is activated by "Fructose-1,6-Biphosphate" & Inactivated by "ATP & Alanine"

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"EMP Pathway" | GLYCOLYSIS | → Cytosolic pathway → occurs in all cells in the body.
→ Production of ATP → Main Purpose

(Mitochondria; O_2) Aerobic → End → Pyruvate
↳ end product of glucose oxidation* ⇓ $CO_2 + H_2O$.

Anaerobic (Lack of Mito-chondria or O_2 or both) → Lactate.

| ATP consumed — HK, PFK |

* Pyruvate — 1st formed — End form

Glucose
ATP → ADP (Mg^{2+}) — glucokinase or Hexokinase
G-6-P
⇅ Phosphohexose isomerase
Fructose-6-P
ATP → ADP — Phosphofructokinase-I ↓ Rate Limiting Enzyme — Committed steps of glycolysis
Fructose 1,6-Biphosphate
⇅ Aldolase (A|B|C) → Isoenzymes
DHAP (3C) ⇄ Phosphotriose isomerase ⇄ Glyceraldehyde 3-Phosphate (3C) — Iodoacetate
$2 \times NAD^+$ → $2 \times NADH + H^+$ ← Pi — Glyceraldehyde 3-P dehydrogenase
1,3- Bisphosphoglycerate [1,3 BPG]
Substrate Level Phosphorylation ↑ (SLP) → $2 \times ADP$ → $2 \times ATP$ (Mg^{2+}) — Phosphoglycerate kinase ← ⊖ Arsenite
3- Phosphoglycerate
⇅ Phosphoglycerate Mutase
2- Phosphoglycerate ← Enolase, Mg^{2+} — Fluoride

Phosphoenolpyruvate
→ (SLP) → $2 \times ADP$ → $2 \times ATP$ (Mg^{2+}) — Pyruvate Kinase
Q 1st Produced → Pyruvate (End)
Spontaneous change
Pyruvate (Keto)
Anaerobic: $NADH + H^+$ → NAD^+ — Lactate dehydrogenase ⇅
Lactate

Teacher's Signature

* Malate shuttle Required for → (1) Aerobic Glycolysis;
(2) Gluconeogenesis

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Irreversible Rx^n → GK / HK, PFK-I, PK	Rest all reactions are Reversible.

- Substrate level phosphorylation (SLP) | oxidative phosphorylation
  - Rare | Major
  - without ETC; $\bar{c}$/out O_2; $\bar{c}$/out NADH, FADH | $\bar{c}$ ETC; $\bar{c}$ O_2
  - only at 3 places
    - 2 glycolysis
      - Phosphoglycerate kinase
      - Pyruvate kinase
    - 1 TCA cycle [Succinate Thiokinase]

- To get the NAD^+ back under Anaerobic glycolysis Lactate is formed. (Pyruvate is converted into Lactate d/t only get the NAD^+ back).

Reduction Rx^n → Pyruvate $\xrightarrow{LDH}$ Lactate

NADP → NAD^+ *generated

$\boxed{\text{Aerobic Req} \rightarrow O_2 + \text{Mitochondria}}$

Energy :-

- 2 ATP
+ 4 ATP
+ 2 NADH (5 ATP) = zero ATP in Anaerobic glycolysis

Total 4 Produced
Net gain → 2 ATP.

5 ATP ↓ if NADH uses MAS or if No Shuttle Mentioned ↓ Total → 9 ATP

3 ATP ↓ if NADH uses GPS → Total → 7 ATP

Net ATP

• **Anaerobic glycolysis** ⇒ occurs in exercising Skeletal Muscle[a], RBCs[a], Lens[a]; Some Region of Retina; Renal Medulla[a], Testis; Leucocytes

ATP	Aerobic glycolysis	Anaerobic glycolysis	Avg. 18 7 8 5 if glycerol phosphate shuttle is used
Total	9/7/10	4	
Net	7/5/8	2	Avg. 10/8 → acc. to older data

* Shuttle ⇒ set of biochemical Reactions

NADH
↓
Transferred from cytosol to mitochondria [across inner membrane of mitochondria] to form ATP.

- Malate aspartate shuttle
  - NADH → $NADH_2$
  - ⇓
  - 2.5 ATP
  - (+) in Liver
- Glycerol phosphate shuttle
  - NADH → $FADH_2$ (FAD+ get H+ in mitochondria Matrix)
  - NADH ⇓ it only give H+ & leave NAD+ in cytosol
  - $FADH_2$ ⇓ 1.5 ATP
  - (+) in Brain; skeletal Muscle

INHIBITORS OF GLYCOLYSIS

Fluoride ⊖ → Enolase — [a] So, Sodium fluoride is mixed with Potassium oxalate, as an anticoagulant in Blood sample for Sugar estimation.

Iodoacetate ⊖ → glyceraldehyde 3P dehydrogenase

Arsenite → affecting the glycolysis, but all not true as this doesn't stop glycolysis.
→ glycolysis continues, but ATP Production is Reduced.

Teacher's Signature

Mutase ⇒ convert one group on one atom - to Another atom in Same compound.

ip → Inorganic Phosphate

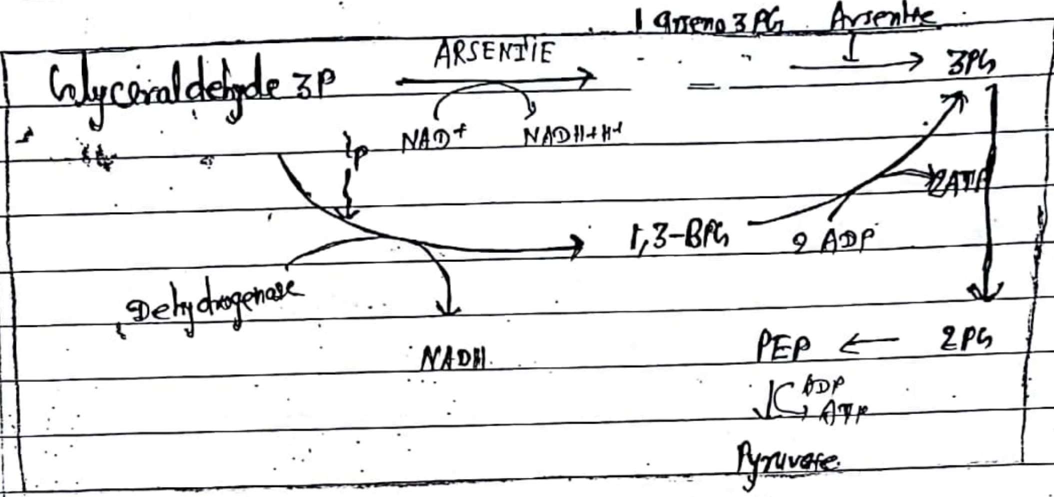
Arsenite competes with ip to take 1st position in compound

↓

Product is 1 arseno 3P instead of 1,3 BPG

↓

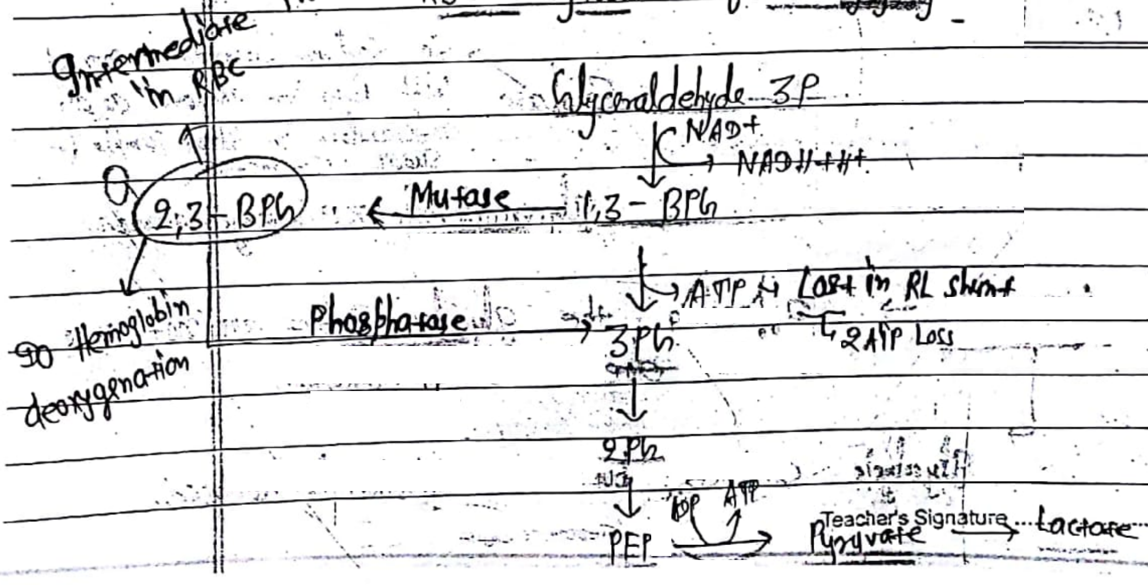
- Breaks to form 3PG (∴ 2ATP is not formed)



| R.L. Shunt | RAPAPORT - LEUBERING CYCLE |

- Inside RBC
- Some steps of glycolysis are shunted.
- There is loss of ATP (2 ATP)
- 2,3 - Bisphosphoglycerate (2,3-BPG) Produced in RBC cytosol from glycolysis.

Produced



Pyruvate Kinase & Aldolase deficiency in erythrocyte -> Hemolytic Anemia

• Pyruvate Kinase & Aldolase deficiency in erythrocyte → Hemolytic Anemia

1 mmole/L = 18 mg/dl

* RL Shunt — ATP Produced = 2; ATP used = 2; Net ATP = 0 { Because NADH → ATP doesn't occur in RBC }

ATP comes from — glucose, FA, KB
↓
*** RBC only depend on glucose for energy.

GLUCOKINASE & HEXOKINASE :-

GLUCOKINASE	HEXOKINASE
• Type IV isoenzyme of Hexokinase	• I, II, III types of isoenzyme
• Found in Liver & β-cells of Pancreas	• Found in all other cells
• Low affinity binding to glucose	• High affinity binding to glucose
• Km High [180 mg/dl = 10 mmole/L] Substrate concⁿ. Means we need high amount (180 mg/dl) of glucose in presence of glucokinase to get 1/2 V_{max}.	• Km Low [0.9 mg/dl] 0.05 mmole/L [affinity $\propto \frac{1}{Km}$] Affinity $\propto \frac{1}{Km}$
• Liver & Pancrease acts (i.e. glucose concn) when glucose is present in ↑ concⁿ.	• Peripheral cells are able to utilize glucose at very less concⁿ [Uninterrupted supply of glucose to brain at night is due to ↑ affinity of HK to glucose]
• Insulin activates GK	• No effect of Insulin on HK

Insulin activate GK

No effect of Insulin on HK

GLUCOKINASE | **HEXOKINASE**

∴ after meal GK acts on food in presence of Insulin in liver & β-cells of Pancreas
[Insulin comes in blood only in presence of ↑ glucose eg → Meal]

• 3 enzymes of glycolysis are activated in presence of insulin because they need dephosphorylated form for their activities

⇓

* Insulin dephosphorylates the enzyme (ID).

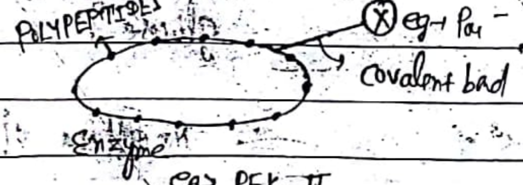
1. Glucokinase
2. Phosphofructokinase – II
3. Pyruvate kinase

* Insulin activates PFK–I by allosteric Modulation

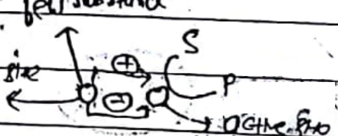
GLUCOKINASE	HEXOKINASE
G-6-P has no effect on GK	G-6-P has negative feedback on HK.

Enzymes

Covalent Modification



Allosteric Modification



• Other than active site, allosteric sites are present on which few things bind which may ⊕ or ⊖ the production. eg → P.F.K – I

Multifunctional enzyme ⇒ One protein; Multiple catalytic activity

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active in PO4 form

PFK-II & Fructose-2,6-Biphosphatase (Bifunctional enzyme)
⇓
One protein two catalytic activity

One acts at a time (decided by Dephosphorylation & phosphorylation).

Insulin — dephosphorylate

→ Liver

⊕ Fructose 6P

PFK-2 — deP form active

Fructose 2,6-Bisphosphate ⊕ PFK-I

F-2,6-BPase — Phosphorylated active

Fructose-6-Phosphate

Fructose-1,6-Biphosphate

GLUCAGON
↓
Phosphorylates
↓
PFK II ⊖
F2,6 BPase ⊕
F2,6 BP↓
by existing F2,6BP converted into F6P
F6P ↑
⇓
glycolysis down Regulates

Fructose 2,6 BP (most imp. allosteric Regulator) of PFK-I

5'-AMP (Energy deficient state)
Insulin
(Fructose-2,6-Bisphosphate) ⊕ → PFK-I

⊕ → Positive allosteric Modulation
⊖ → Negative allosteric Regulator

Regulator

ATP ⊖
Citrate ; b/c citrate Indicates the acetyl co-A
Proton
Glucagon

Insulin ↑ synthesis of F-2,6-BP → Glycolysis ↑
Glucagon ↓ses " " "

Q. Decreased glucose in hepatic cells triggers all except →
i) Glucagon ↑ ; ii) ⊕ of F2,6 BPase ; iii) ⊖ of PFK-2 ; iv) F,2,6 BP ↑

Teacher's Signature

PASTEUR EFFECT

→ Inhibitory effect of O_2 on glycolysis

⇓

It is due to ↓ AMP/ATP ratio.

AMP has ⊕ve effect on PFK-1, So, ↓ AMP causes inhibition of glycolysis.

→ $\boxed{\uparrow O_2 \longrightarrow \uparrow \text{oxidative phosphorylation} \rightarrow \downarrow \text{AMP } (\uparrow \text{ATP})}$

CRABTREE EFFECT

→ Relative Anaerobiosis produced; when glucose concⁿ is increased in constant or limited supply of oxygen..

⇓

Lactate ↑

→ Glucose $\xrightarrow[\text{used}]{O_2}$ Aerobic glycolysis

↓ Later → No O_2 for ↑ed glucose

Anaerobic glycolysis

↓

Muscle Pain ⟵ Lactate ↑↑ [Anaerobiosis]

CONVERSION OF PYRUVATE TO ACETYL CO-A

Pyruvate — Cytosol

→ Pyruvate-proton symport system

Proton ← Pyruvate — Mitochondrial Matrix

Pyruvate Dehydrogenase complex

⇓

5 enzymes

CO_2 ←

NADH ← Acetyl co-A

Required for this ← this always give ATP, No shuttle

→ Pyruvate is transported to mitochondrial matrix via Pyruvate – proton Symporter

PDH complex → Pyruvate Dehydrogenase complex

Enzymes	Co-Enzymes
→ Pyruvate dehydrogenase / carboxylase	→ Thiamine (B_1) Pyrophosphate
→ Dihydrolipoyl transacetylase	→ Lipoic acid
→ Dihydrolipoyl dehydrogenase	→ CoA (B_5 / Pantothenic acid)
	→ FAD (B_2 / Riboflavin)
	→ NAD (B_3 / Niacin)

It's deficiency ⇐ Pyruvate dehydrogenase
⇓
Most common cause of congenital LA (Lactic acidosis)

Pyruvate dehydrogenase ↓ 1st enzyme ↓ active in Dephosphorylated form.

Regulation of Pyruvate dehydrogenase

PDH (Active) → PDH kinase → PDH-(P) (Inactive)

(+) Ca+, Mg+2, insulin → PDH (Active)

(+) $\frac{\text{Acetyl CoA}}{\text{CoA}}$, $\frac{\text{NADH}}{\text{NAD}^+}$, $\frac{\text{ATP}}{\text{ADP}}$ Ratio → PDH kinase

(−) Acetyl CoA, NADH, ATP, Glucagon → PDH (Inactive)

if ↑ ⇒ PDH kinase (+) → PDH complex will not act

e.g. → NADH ↑ (>1) in Mito matrix
↳ PDH complex will not act

→ each acetyl Co-A produces ⇒ 10 ATP.

→ Total No. of ATP formed by complete oxidation of glucose in aerobic condition
⇓
→ 32 (9 + 5 + 20 − 2)
9 → glycolysis
5 → Link Rxn
20 → TCA

→ 38 (older calculation) [10+6+24]−2

F A M are:
F → Fluoroacetate
A → Arsenite
M → Malonate

F, A → Non-competitive Inhibitor
M → competitive Inhibitor

F ↓ A (Aconitase)
A ↓ K (α-KG Dehydrogenase complex)
M ↓ S → Succinate dehydrogenase

Vitamins in TCA cycle → ① Riboflavin → FAD
② Niacin → NAD
③ Thiamine → α-Ketoglutarate dehydrogenase
④ Pantothenic acid → Acetyl Co-A

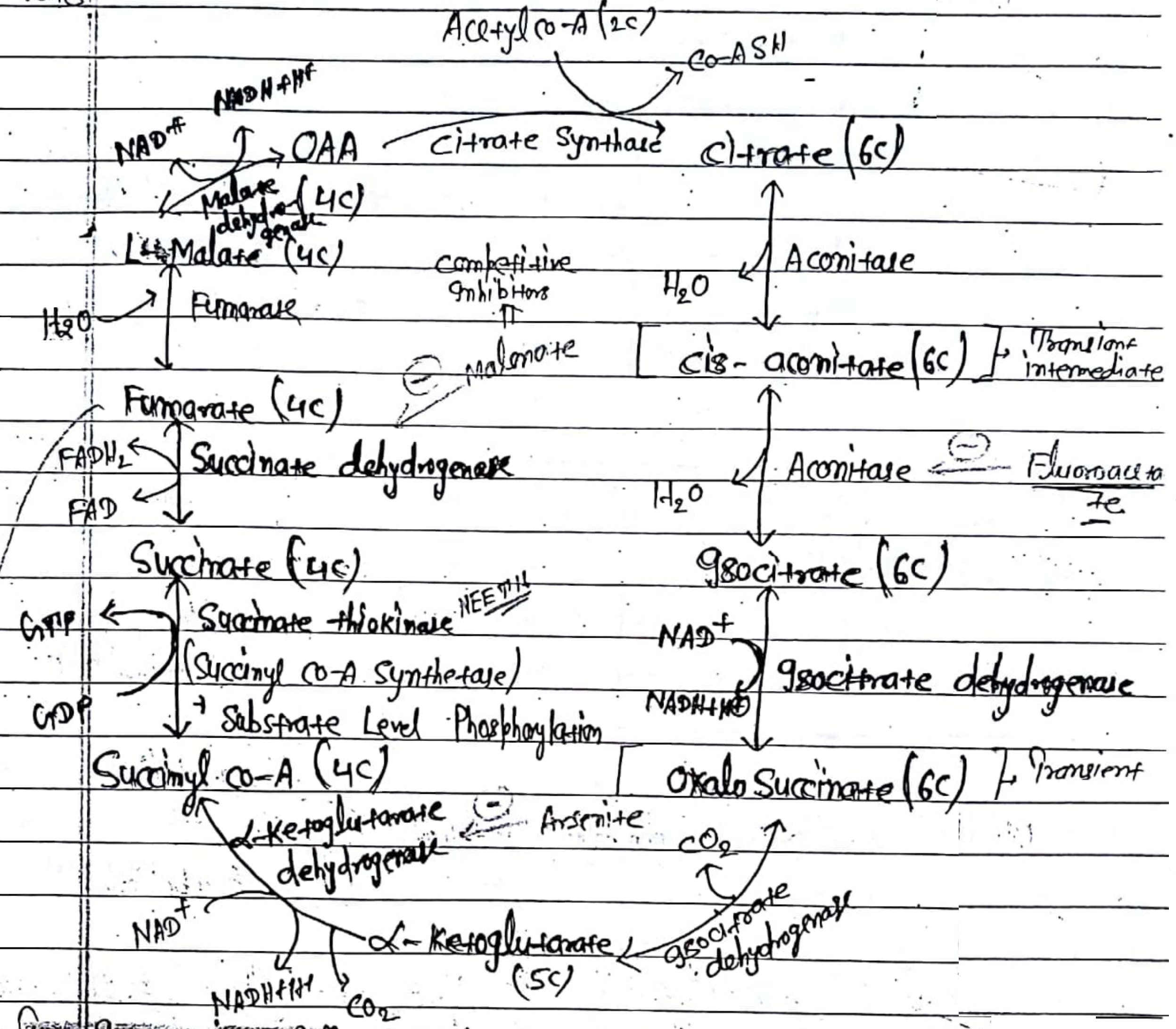
Pyruvate ↓ Source of 1st substrate

TCA CYCLE

Mitochondrial Rx^n

coenzyme of TCA → NAD^+

RBC → No mitochondria ↓ No TCA



• Two irreversible Rx^n → ① oxaloacetate to citrate; ② α-KG to Succinyl Co-A

Two irreversible Reactions

TCA cycle

Isocitrate dehydrogenase → Rate limiting enzyme.

Citrate synthase — As such No Rate limiting Enzyme

Total ATP = 3 NADH
1 FADH
1 ATP

$\boxed{10\ ATP}$ → 1 cycle — In older calculation ⇒ 12 ATP

Derived from Tyrosine & Phenylalanine

Teacher's Signature ....

Hemolytic Anemia → due to deficiency of → Pyruvate kinase
→ Aldolase A

Allosteric Regulation in TCA cycle

Enzyme	Positive alloster (↓ Energy state)	Negative alloster (↑ Energy state)
Citrate Synthase	ADP	ATP NADH Succinyl CoA Fatty acyl CoA
2. Isocitrate dehydrogenase	ADP Ca^{2+}	ATP NADH
3. α-Ketoglutarate dehydrogenase	Ca^{2+}	ATP GTP NADH Succinyl CoA

TCA cycle → Amphibolic pathway → Catabolism + Anabolism

→ Final common pathway of Fat, carbohydrate & protein Metabolism ⇒ TCA cycle.

→ Total (4) Dehydrogenase enzymes in TCA cycle.

→ Mitochondrial matrix → site of all enzyme
except → Succinate dehydrogenase
↓
Inner Mitochondrial membrane

Teacher's Signature ....

13

Urea formed in → Liver; Brain; Kidney

Selective Non-carbohydrate Precursor ⇓ Pyruvate; Lactate; Propionyl Co A; Fumarate; glycerol; Aacids (glucogenic Alanine)

→ New glucose formation from Non-carbohydrate Precursor

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Mainly in (Liver) ⇓

GLUCONEOGENESIS → In Liver; Kidney; GIT

Acetyl CO A, Fatty acids, Ketone bodies } → are not substrate for gluconeogenesis

mainly in cytosol, Some substantial Role ⇓ precursors are produced in mitochondria.

Role of Insulin on carbohydrate metabolism [↓ glucose in Blood]

- i) ↑ glycolysis (liver);
- ii) ↓ gluconeogenesis;
- iii) ↑ glucose uptake by cell;

GLUT4 receptor on these organs ↓ on starvation

GLUT4 → depends on insulin for its activity → (+) on Skeletal muscle, Adipose tissue, Heart } depend on insulin for glucose uptake

- iv) ↑ glycogenesis
- v) ↓ glycogenolysis.

* Insulin Repress the gene of Pyruvate carboxylase

GLUCONEOGENESIS ⇒ Glucose is synthesized from Non-carbohydrate precursors

*** Should be part of TCA cycle / glycolysis or have property of converted to TCA cycle / glycolysis intermediate — glucogenic AA [Alanine]Q ⇓ 18 AA

Q All AA Except Leucine & Lysine are glycogenic AA

Pyruvate Q

Lactate Q

Fumarate

Propionic acid [Propionyl CoA]Q

glycerol [alcohol]Q

* By Pyruvate to produce glucose in gluconeogenesis; Reversible steps of glycolysis are used in Reversed Manner & Irreversible steps of glycolysis are by-passed by different & dedicated enzymes of gluconeogenesis

∴ Acetyl CO-A & Substrate giving acetyl coA [FA & KB] → can't make glucose

* odd no. C-atoms give Propionyl-CoA → Succinyl CoA → TCA intermediate

comes from valine; ←

* Glucose-6- Phosphatase -
* Glycerol produce glucose by formation of DHAP by glycerol kinase & glycerol dehydrogenase (Page 24)

Glucose

AA

PEP(3C) Pyruvate (Cytosol)

Kinase

[GTP] PEP carboxy-kinase

CO_2

decarboxylating enzyme

mitochondria

Pyruvate $\xrightarrow{\text{PDH complex}}$ Acetyl CoA

Pyruvate(3C)

CO_2 → Pyruvate ← (+) allosteric

ATP, Biotin: Pyruvate Carboxylase

OAA (4C)

OAA(4C)

Acetyl CoA

[Fatty acid oxidation]

Malate DH: NADH → NAD+

NADH → Malate DH → NAD^+

Malate(4C) ← Malate(4C)

Here, Reducing equivalents from mitochondria to cytosol are transported by Malate Q

→ In gluconeogenesis →

PFK-1 counter part → Fructose 1,6 Bisphosphatase Q

GK/HK counter part → Glucose 6 Phosphatase Q

Pyruvate kinase counter part → Pyruvate Carboxylase Q

→ organs involved in Gluconeogenesis → Liver (most imp); Kidney; GIT.

V.V.Q. AIIMS May (17)

Chemical Rxn that form intermediate of Metabolic Pathway.

eg If Anaphylatic Reaction [Killing up reaction] ----> Filling TCA intermediates

Q L-methyl malonic Acidurua or L-methyl malonyl uria

Valine

↓

Propionic acid (3C) [Propionyl CoA]

Biotin, ATP: Propionyl CoA carboxylase

↓

D-methyl malonyl CoA (4C)

↓ Racemase

L-methyl malonyl CoA (4C)

def of B12 enzyme

Succinyl CoA $\xleftarrow[(B_{12})]{\text{Mutase/Isomerase}}$ L-methyl malonyl CoA (4C)

Teacher's Signature ....

Succinyl coA comes from → V → Valine
I → Isoleucine
M → Methionine

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eg:- α-KG + NH_3 ⟶ glutamate

~~citrate ⟶ FA synthesis~~

→ Cataplerotic rx^n are kya "Emptying Rx^n"
↳ TCA Intermediate ⟶ Non-TCA Intermediate

→ Most imp. Anaplerotic rx^n in human
↓

Pyruvate $\xrightarrow{\text{Pyruvate Carboxylase}}$ OAA
↓
TCA intermediate

Pyruvate carboxylase → under Repression in presence of Insulin
↓
Not formed
↓ gluconeogenesis

Insulin
↓
dephosphorylates

glycolysis ↑es

F6P $\xrightarrow{\text{PFK-2}}$ $\boxed{\text{F 2,6 BP}}$ ⟶ PFK-1 (+)

F 2,6 BP $\xrightarrow{\text{F2,6 BPase}}$ F6P

F6P ⇅ F1,6 BP

F,1,6 BPase ← (+) ← citrate

$\boxed{\text{Glucagon} \downarrow \; \downarrow \text{Glycolysis} \; \uparrow \text{Gluconeogenesis}}$

→ Human brain consumes about 120gm of glucose/day out of the 160gm needed by the body.

NEET'16 * Rate Limiting enzyme of gluconeogenesis ⇒ Fructose 1,6 - Bisphosphatase

* Starvation } 2 cond^n where gluconeogenesis takes place; b/c of Lack
Diabetes } of Insulin.

* Regulating enzyme of gluconeogenesis ⇒ ① Pyruvate carboxylase
② PEP carboxykinase
③ Fructose 1,6 Bisphosphatase

Date
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FRUCTOSE METABOLISM

Q. Part of F-1-P goes to Ketose (DHAP)

FK | HK not isoenzymes
but GK | HK all isoenzymes

Fructose
↓ Liver (Major) | ↓ Skeletal muscle, Adipose

Liver (Major):
Fructose $\xrightarrow[\text{ATP} \to \text{ADP}]{\text{Fructokinase}}$ Fructose-1-P

Fructose-1-P $\xrightarrow{\text{aldolase B}}$ DHAP (3C) (Ketose) + Glyceraldehyde (3C)

Glyceraldehyde $\xrightarrow[\text{ADP} \to \text{ATP}]{\text{Triokinase}}$ Glyceraldehyde 3P → GLYCOLYSIS

DHAP → GLYCOLYSIS

the phosphate of Fructose-1-P of ketose goes to side

Skeletal muscle, Adipose:
Fructose $\xrightarrow{\text{Hexokinase}}$ Fructose-6-P → GLYCOLYSIS

PFK-1 → No Role of fructose metabolism in Liver
↓
Insulin has no role in fructose metabolism
↓
Diabetic Patient will very well utilize Fructose

↑↑ Fructose (Excess fructose diet) → ↑ glycolysis → ↑↑ Pyruvate
↓
↑↑ Acetyl CoA
↓
↑↑ Fatty acid Synthesis in liver
↓
Fatty Liver

↑↑ Fatty acid Synthesis → ↑VLDL in Blood → LDL in Blood → Atherosclerosis

Teacher's Signature

• Highest concn of Fructose Among biological fluids is found in → Seminal fluid.

Date
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∴ Excess fructose diet is "Atherosclerotic"
⇓
Not advisable to (N) Person also

→ Deficiency of Fructokinase
↳ Essential/Benign Fructosemia

→ Aldolase B deficiency
↳ Fructose 1-P accumulate in liver
⇓
K/as "Hereditary fructose Intolerance" [HFI]
⇓
Baby ⇒ Lethargic
→ Vomiting, diarrhoea after fructose rich diet ;
Death c̄ in the 5years
→ Hypoglycemia (episodic)
→ ↑ Uric acid in blood
→ ↑ lactic acidosis
→ Hepatomegaly

⇒ Fructose 1P $\xrightarrow{\ominus}$ Glycogen phosphorylase
↓
Maintains blood glucose level
[∴ Baby will have hypoglycemic episodes as rate limiting enzyme of glycogenolysis is deficient]

⇒ Excess fructose-1P is formed ; which leads to consumption of ATP in liver ↓. [↓ATP ⇒ ↑glycolysis]
, b/c it sequestrate the inorganic phosphate

↑ Glycolysis c̄ limited O_2 → Crabtree → Lactic acidosis

Teacher's Signature ....

* Alcohol $\xrightarrow[\text{dehydrogenase}]{\text{Alcohol}}$ Acetaldehyde $\xrightarrow[\text{dehydrogenase}]{\text{Acetaldehyde}}$ Acetic acid

NAD⁺ → NADH   NAD⁺ → NADH → it converts Pyruvate → Lactate → Lactic acidosis → Hyperuricemia

Date
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Gluconeogenesis is also downregulated b/c Pyruvate is Not available
⇓
Fasting Hypoglycemia

↑ Lactic acidosis leads to ↓ uric acid excretion from the kidney
⇓
Hyperuricemia

⇒ ATP ⊖ → "Denovo Purine Nucleotide Biosynthesis"

⇒ In hereditary fructose intolerance → ATP ↓
⇓
∴ ↑↑ Purine nucleotide
⇓
↑ Uric acid.

⇓
It is a lethal condition
⇓
Baby doesn't survive > 5 yrs
⇓
Early death seen

POLYOL / SORBITOL PATHWAY

→ doesn't occur in liver.
→ Seen in → Lens
Peripheral Neurons
Basement membrane of glomerulus
Testis; sperm → Hygroscopic alcohol
⇓
Absorb water

→ GLUCOSE $\xleftrightarrow{\text{Aldose Reductase}}$ Sorbitol
↓ Sorbitol dehydrogenase
Fructose

*→ Occur when blood glucose level is very high (eg→ DM)

→ Excessive sorbitol pathway is one reason for
- Diabetic Cataract; GlomeruloNephritis
- Diabetic Neuropathy etc

Teacher's Signature ....

↑ Sorbitol → ↑ water absorption [hygroscopic]
↳ Causes "Blurring of vision".

→ Aldose Reductase → Target enzyme to ↓ DM complications.
↓
If inhibited → No Sorbitol formed.

Galactose ⇒ Dietary Non-essential
Need to → ATP, Ganglioside

[GALACTOSE METABOLISM]

Almost all galactose is utilized in → Liver.

Galactose
ATP → ADP (Galactokinase)
↓
Galactose 1-P
UDP-glucose → UDP galactose (Galactose-1P uridyl transferase; Epimerase)
↓
Glucose-1-P-
↓ Phosphogluco-Mutase
Glucose 6P → Glycogen
↓ → HMP-Shunt
glycolysis → Uronic acid

- At some steps epimerization takes place.
- Galactose is converted to glucose.

* UDP-glucose is Must for Galactose Metabolism.

⇒ In the case of galactokinase deficiency
↳ Benign Galactosemia

→ In galactokinase deficiency

⇓

Galactose ↑↑

Aldose Reductase → Galactitol / dulcitol

$NADPH + H^+$ → $NADP^{\oplus}$

↓

Hygroscopic

↓

accumulates in Lens

⇓

Cataract

• Baby born c̄ Cataract.

→ Galactose 1P uridyl-trans-ferase deficiency

⇓

Classical galactosemia

Rare Congenital disease
Autosomal Recessive disorder

↓

Galactose 1P accumulated → Hepatotoxic

↓

Baby has →
- Cataract
- Organomegaly
- Hypoglycemia
- Mental Retardation
- Lactic acidosis
- Hyperuricemia

** Phosphorylated Sugar [glucose, galactose] can't cross the cell membrane → accumulate in same cell

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→ energy consumed in its formation → ↓ATP → ↑glycolysis → LA → Hyperuricemia

→ accumulates in liver → [Hepatomegaly]

Galactose 1P —(−)→ Glycogen phosphorylase
↓
Episodes of hypoglycemia
↓ Repeated steps
Leads to Mental Retardation.

Rx of Galactosemia → Galactose free diet (complete Restriction)

→ Galactose is dietary Non-essential (i.e. can be formed in Body) ↓ from glucose
↓
Required for ganglioside formation (Nerve sheath)

** AIIMS Nov 16: Presence of Reducing material (galactose) in urine c̄ Negative glucose oxidase test suggests diagnosis of galactosemia

→ GLUCOSE
↓
GLUCOSE-6P
↓ Phosphoglucomutase
GLU-1-P
↓ UDP-glucose Pyrophosphorylase
UDP-glucose
↓ 4 epimerase
UDP galactose

UDP galactose + glucose —Lactose synthase→ Lactose

** Lactase (enzyme of β-galactosidase group): Lactose → Glucose + Galactose

Galactose → used for ganglioside synthesis in Nerve sheath

if 4-epimerase deficient in Liver
⇓
Dual galactosemia
⇓
can't be treated. ∵ Not told to take galactose free diet; b/c Nerve sheath should form

∴ Before advising diet restriction c̄ galactose we need to check for intact 4-epimerase. ***

"HMP shunt doesn't Produce energy".

→ Galactosemia $\bar{c}$ 4 epimerase deficiency can't be treated as galactose can't be synthesized in body for ganglioside synthesis

• cytosolic — **HMP PATHWAY** → No ATP Production

① → NADPH Produced ; ② → Ribose 5P — for Nucleotide Synthesis

∴ Hexose monophosphate Pathway / Pentose phosphate Pathway [PPP]

→ Occurs in → Liver
- Adrenal gland
- Mammary gland (Lactating not the Non-lactating)
- RBC → To Protect RBC
- Placenta
- Testis, ovary
- Adipose tissue

glucokinase is not a committed step - irreversible

committed step - form product for 1 pathway

committed step → irreversible phase

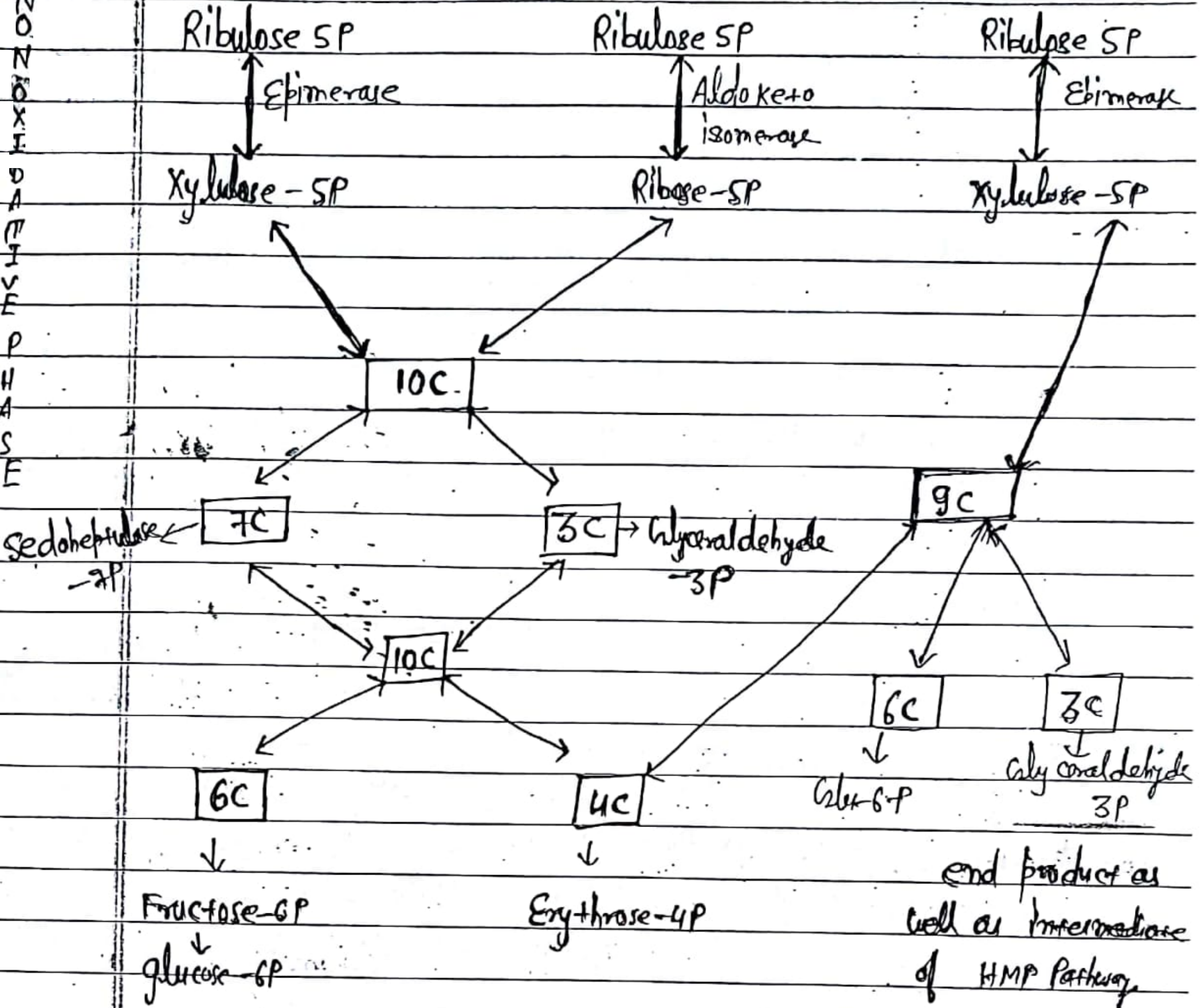
→ Starts with 3 molecules of glucose

GLUCOSE	GLUCOSE	GLUCOSE
↓ Glucokinase	↓ GK	↓ GK
Glu-6-P	Glu-6-P	Glu-6-P
↓ $NADP^+$ → NADPH; G6PD → Rate limiting enzyme (committed step)	↓ $NADP^+$ → NADPH; G6PD	↓ $NADP^+$ → NADPH; G6PD
6-phosphogluconate	6-Phosphogluconate	6-Phosphogluconate
↓ $NADP^+$ → NADPH; 6 Phosphogluconate Dehydrogenase; CO_2	↓ $NADP^+$ → NADPH; 6PGDH; CO_2	↓ $NADP^+$ → NADPH; 6PGDH; CO_2
Ribulose-5-phosphate (5C)	Ribulose-5-phosphate (5C)	Ribulose-5-phosphate (5C)

Teacher's Signature ....

Reversible Rxn

NON OXIDATIVE PHASE



5C → Ribose-5P ⇒ having biologically imp. to form Nucleotide synthesis
— Ribulose-5P
Xylulose-5P

Intermediates → Glyceraldehyde -3P (3C)
Erythrose -4P (4C)
all 5C compound
Fructose -6-P
Sedoheptulose -7P

Teacher's Signature ....

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- Pyruvate $\xrightarrow[\text{(TPP)}]{\text{PDH}}$ Acetyl Co-A ⇒ Pyruvate utilized in presence of B_1

- Thiamine deficiency causes ↓ energy production as it is coenzyme of PDH ~~(Required for glucose breakdown)~~.

3 Glucose-6P + 6NADP$^+$ + 6H$^+$

↓

2 Glucose-6P + 6 NADPH + 3CO_2 + Glyceraldehyde - 3P

→ Continuous condensation & dissociation in HMP Pathway is catalysed by → Transaldolase, Transketolase → Req. in Reversible phase

PLP (vit B_6) → B_1

Pyridoxal phosphate (PLP) → Req. for Transaldolase activity

→ To find out vit B_1 deficiency → Transketolase activity in RBC is assessed

↓

earliest manifestation

↓

↓ Transketolase activity

* Hemolytic Anemia is d/t ⇒ deficiency of
  - G6PD (96%)
  - Pyruvate Kinase (4%)
  - Phosphoglucomutase (~1%)

ROLE OF NADPH → (1) Reductive Biosynthesis of fatty acids & steroids [Pathway requiring H$^+$] → For Reducing Source - NADPH

(2) Tackle the oxidative stress in a cell via. Reduced GSH

(3) Maintain the Reduced form of glutathione [GSH]
- → Co-enzyme activity
- → AA transport across Membrane
- → Tackle oxidative stress

(4) at active site of enzyme.

Teacher's Signature

superoxide O_2^- (More toxic) $\xrightarrow{\text{Superoxide dismutase (SOD)}}$ H_2O_2 (Less toxic) $\xrightarrow{\text{Fenton's Rxn}, Fe^{++}}$ OH^- (Most potent free Radical)

H_2O_2 $\xrightarrow{\text{Glutathione Peroxidase, Catalase}}$ H_2O

Source of NADPH → ① HMP Shunt (Major > 90%);
② Malic enzyme
③ Isocitrate dehydrogenase (cytosolic)

→ Role of NADPH as an Antioxident (free-Radical Scavenging) reactions →

H_2O_2 ; 2GSH (Reduced) → Glutathione Peroxidase → $2H_2O$; G-S-S-G (oxidised)

Selenium containing enzyme

G-S-S-G (oxidised) ; NADPH+H+ → Glutathione Reductase → 2GSH (Reduced) ; NADP+

NADPH+H+ → Source of H+

FAD containing enzyme

* In G-6-PD deficiency: hemolysis seen b/c it can't tackle the oxidative stress.
∴ Reduced GSH converts $H_2O_2 \rightarrow 2H_2O$ by giving its H+ & NADPH maintains GSH in Reduced State

H_2O_2 → Free Radical → Needs to be Scavenged.

AIIMS

ENZYME	COVALENT MODIFICATION: ACTIVATOR	COVALENT MODIFICATION: INACTIVATOR	ALLOSTERIC MODIFICATION: STIMULATOR	ALLOSTERIC MODIFICATION: INHIBITOR
• GLYCOGEN SYNTHASE →	Insulin	Glucagon (in Liver) Epinephrine (in Liver & Muscle)	Glucose-6-Phosphate	
• GLYCOGEN PHOSPHORYLASE →	Glucagon (in Liver) Epinephrine (in Muscle)	Insulin	AMP (in Muscle) Glucose (in Liver) Ca^{2+} (in Muscle)	ATP (Liver & Muscle) Glucose-6 (in Muscle)

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Not Random; eccentric Molecule

GLYCOGEN METABOLISM

- Storage form of glucose In Animals.
  - Liver ⇒ 4% of Liver mass → 72 gm
  - Muscle ⇒ 0.7% → 245 gm
  - Extracellular ⇒ 0.1% → 10 gm
- Max^m glycogen → acc. to % or w/w → Liver
  - acc. to total weight → Muscle

GLYCOGENESIS → In cytosol

Synthesis of glycogen from glucose is "GLYCOGENESIS".

Nucleotide, glucose essential for → UDP-glucose

Glucose
↓ GK (ATP → ADP)
Glucose-6-P
↓ Phosphoglucomutase
Glucose-1-P
↓ UDP-glucose Pyrophosphorylase (UTP → PPi)
UDP Glucose
↓
- Nucleotide glucose*
- Glycogen synthase
- Branching Enzyme

↓
GLYCOGEN

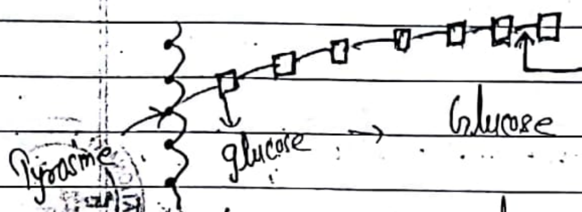
UMP + PPi
PPi = Pyrophosphate
UMP → combine to G-1-P to form UDP glucose

Glycogenin → Protein
Glycogenin → Receive glucose from the glucose

Q

Glucose 1P + UMP → UDP-glucose

UMP ↓ comes from UTP.



α-1,4-glycosidic Bond

Tyrosine — glucose → Glucose is 1st added to Tyrosine

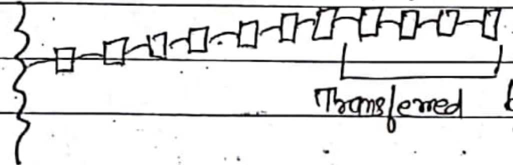
Glycogenin → glucose transferred to form a linear chain of 7 glucose by

(Protein)

Many AA

↓

glucosyl transferase [α 1,4 Bond]



8 9 10 11

↦ Rate Limiting enzyme

Transferred by glycogen synthase [α 1,4 Bond]

Glycogen Primer [Glycogenin + 7 glucose residues]

↓ Branching enzyme → cleaves outer 6 glucose Residue

** [Amylo (1,4→1,6) transglucoside activity]

or

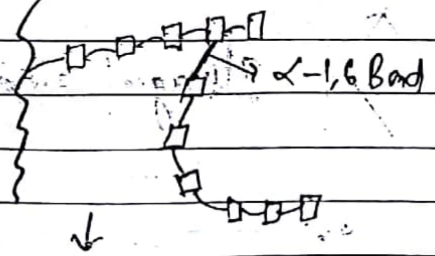
** [Amylo (1,4→1,6) glucan transferase activity]

↓

Now hexasaccharide is transferred in a Branch form

↓

Breaks 1,4 bond & forms α 1,6 Bond



→ α-1,6 Bond

Breaks 1,4 bond & forms
Alfa 1,6 Bond

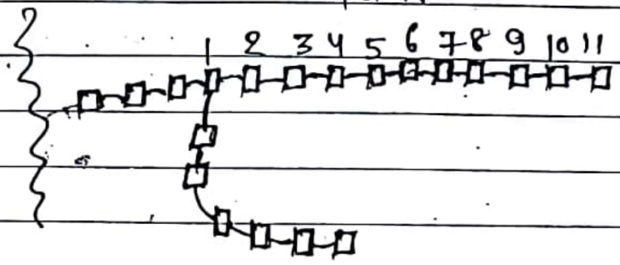
↓

↓

Glycogen Synthase further acts to add glucose residues to form Linear chain (α-1,4 Bond)

↓

'Add 11 Residues of glucose from Branching point.



GLYCOGEN SYNTHASE → active in Dephosphorylated form

↓

∴ Insulin is needed.

GS (active) —Protein kinase→ GS-(P) (Inactive)

↓

CAMP dependent

K*

Glucagon (+) → CAMP ← (−) Insulin
Epi, Norepi

ATP
↓ Adenyl cyclase
CAMP

(−) Insulin

(+) glucagon / epinephrine / NE

→ Most enzymes are → Monofunctional (1 active site)

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GLYCOGENOLYSIS → cytosolic

Important Enzymes →

GLYCOGEN PHOSPHORYLASE	BIFUNCTIONAL ENZYME
Rate limiting enzyme of glycogenolysis.	- 2 catalytic activity - 2 active sites
• Active in Phosphorylated form	Trisaccharide transferase activity → a/k/as "Amylo [1,4→1,4] glucan transferase" or (1,4→1,4)-transglucosidase
AIIMS Nov'17 Needs (PLP) for action [B_6] ↳ Co-enzyme [Major PLP is present for muscle glycogen phosphorylase]	Debranching activity → a/k/as "α-1,6-glucosidase" ⇓ cuts 1,6 Bond • Release free glucose
• Needs Inorganic Phosphate for its activity.	
• a/k/as "α-1,4-glucosidase" ⇓ cut 1,4 Bond	
• Release glucose-1-P	

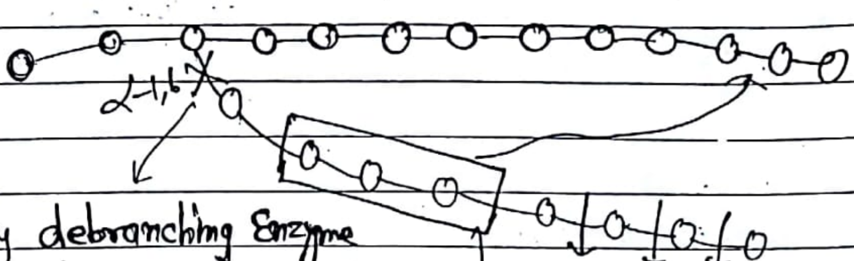
AI→08 Glycogen is degraded to "glucose-6-phosphate" in muscle & to glucose in liver; b/c Glucose-6-phosphatase is absent in Muscle therefore, Glucose-6-phosphate can't be degraded to free glucose in Muscle.

AI→09 Liver glycogen (Glycogenolysis) & gluconeogenesis serve to Maintain blood glucose levels during overnight fasting.

blood glucose levels during overnight fasting.

Teacher's Signature ....

* Liver glycogenolysis ⇒ during starvation & exercise
* Muscle glycogenolysis ⇒ during exercise



③ Cut by debranching Enzyme
↓
glucose released as free glucose in blood

② Cut & transferred by Trisaccharide transferase
↓
transfer the trisaccharide at terminal end & added in linear fashion.

① cut by glycogen phosphorylase
↓
to give glu-1P
↓
acts till 4 glucose remains in Branch
↓
Glucose-1-P can't cross membrane
↓
∴ needs to be converted to glucose to maintain Blood glucose.

→ Glycogenesis & glycogenolysis is a cyclical process.

Reversible enzyme
common to both glycogenesis & glycogenolysis

Glucose 1P → Phosphoglucomutase → Glucose 6-P

Glucose-6P translocase ← encoded by "SLC37A4" gene
↓
transfers G6P to ER*

Glucose-6-Phosphatase (ER) **
↓
Glucose
↓
* in blood

Liver glycogen → Instant Source of Blood glucose.

G-6-P → Common to Many Pathways

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→ GLUCOSE-6 PHOSPHATASE → deficient in "Von-Gierke disease"

↳ Mainly prt. in liver, kidney & intestine

↳ abt. in Muscle & Brain;

⇓

So; end product of glycogenolysis in muscle

⇓

glucose-6 Phosphate

⇓

~~Muscle uses it for energy via glycolysis.~~

→ due to lack of glu-6-phosphatase

↳ Muscle glycogen is not responsible to maintain Blood glucose.

PK(a) → active phosphorylated form.

→ Glycogen phosphorylase $\xrightarrow{\text{Phosphorylase Kinase}}$ $\boxed{GP}$ (P)

Phosphorylase Kinase needed in active form

[Inactive] - GP(b) → [active] - GP(a)

Insulin → Dephosphorylated | Phosphorylated form, ↑ Glucagon

PK(b) $\xrightarrow{\text{CAMP dependent}}$ PK(a)

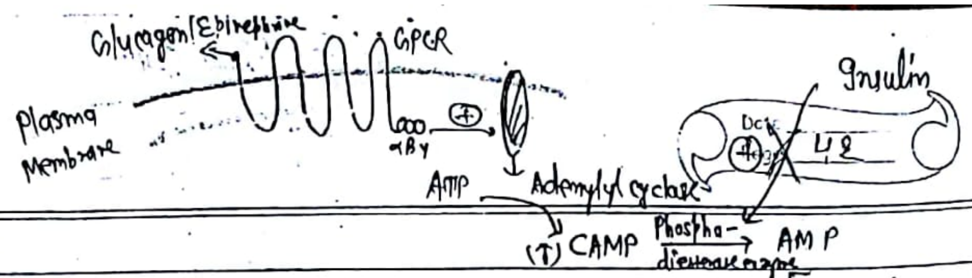
Inactive phosphorylated Protein Kinase active Phosphorylated

↑(+)

(+) → CAMP ← (−)

Glucagon; Epinephrine Norepinephrine | Insulin.

Teacher's Signature ....



⇒ Phosphorylase kinase – Tetramer [4 Subunits]

(α, β) (γ) (δ) → Bind to Ca^{+2}

Bind with Pa^-

catalytic subunit

Calmodulin like subunit.

AIIMS Nov 17 ⇒ Glycogen phosphorylase

Liver	Muscle
↓	↓
Monomeric	Tetrameric
↓	↓
needs 1 PLP	needs 4 PLP
affected by Insulin, glucagon, epi, nor-epi	affected by epi, Nor-epi
↓	↓
Maintains Blood glucose level	Q doesn't maintain blood glucose

AI-99 Stress → epi, Nor-epi ↑ → Liver, muscle → glycogenolysis ↑

⇓

↑ Blood glucose due to Liver glycogenolysis

r's Signature ....

GLYCOGEN STORAGE DISEASE

TYPE	NAME	ENZYME Def.	C/F
0	—	Glycogen Synthase	Hypoglycemia; Hyperketonemia; early death.
Ia	Von Gierke's disease	Q Glu-6-Phosphatase	Q Hypoglycemia; Lactic acidosis; Ketosis; Hyperlipidemia.
Ib	—	ER Glucose-6P transporter (Glu-6P-translocase)	as type Ia Neutropenia; Recurrent infection
II	POMPE's disease	Lysosomal 1,4 and 1,6 glucosidase (Acid maltase)	· Glycogen accumulates In lysosomes in almost all tissue. ① Juvenile onset variant - "Muscle hypotonia" (death from Heart failure by age of 2yr). ② Adult onset variant:- - Muscle dystrophy. (No death)

Teacher's Signature ....

VON-GIERKE'S DISEASE → Hepatocytes

Glycogen

glycolysis ← Glucose-6P → HMP → Ribose 5P → Purine Nucleotide ↑↑ → Uric acid ↑↑ → Hyperuricemia

Glucose-6P ✗→ Glucose

↓ No Blood glucose → Hypoglycemia

Excess glycolysis
c̄ limited O_2 → Lactic acidosis → Hyperuricemia

Hypermia

Hypoglycemia → glucagon secreted → Phosphorylation of many enzymes
↓
also "Hormone Sensitive Lipase"
↓
active in phosphorylated form

Hormone Sensitive lipase
HSL → Mobilize fat from adipose tissue to Blood

Adipose cell
↓
Triacylglycerol → Fatty acid release in Blood → Hyperlipidemia

Hyperlipidemia

Excess β-oxidation in liver
↓
Ketosis → Ketone body formation

Ketosis

Teacher's Signature ....

Liver disease → $\frac{AST}{ALT} < 1$; II, V, VII → Muscle disease

Muscle disease → $\frac{AST}{ALT} > 1$;

IIIa	Limit dextrinosis, Forbe's or cori's disease	* Liver & Muscle debranching enzyme (amylo α 1,6-glucosidase)	· Fasting hypoglycemia · Hepatomegaly in infancy · accumulation of limit dextrin. · Muscle weakness
IIIb	Limit dextrinosis	* Liver debranching enzyme	As type IIa, but no muscle weakness
IV	Amylopectinosis (Anderson's disease)	Glucosyl 4-6 transferase (Branching enzyme)	· Hepatosplenomegaly · accumulation of poly-saccharide c̄ few Branch points.
V	McArdle's disease (type V glycogenosis)	Muscle glycogen phospho-rylase	· Poor exercise tolerance, · Muscle glycogen accu-mulates → abnormally high (2.5-4%) · blood lactate very low after exercise
VI	Her's disease	Liver glycogen phospho-rylase (Hepatic)	· Hepatomegaly · accumulation of glycogen in liver · Mild hypoglycemia

Teacher's Signature ....

Lactose intolerance → Lactase deficiency (β-galactosidase)

VII	Tarui's disease	Muscle & erythrocyte Phosphofructokinase-1 ↓ No glycolysis ↓ ↓ glycogenolysis (G-6-P ... the glycogenolysis) (Muscle fatigue)	Same as V; also hemolytic Anemia; Muscle fatigue
VIII	—	Liver phosphorylase kinase	· Hepatomegaly · accumulation of glycogen in liver · Mild hypoglycemia · good prognosis
IX	—	Liver & Muscle phosphorylase kinase.	· Same as VIII · Muscle also involved
X	—	CAMP dependent protein kinase A	· Hepatomegaly · accumulation of glycogen in liver

Mnemonics :-

Vo	Physics	Chem	Aur	Maths	Hathiyar	Thi
Von	Pompe's	Cori	Andersen's	McArdle	Her's	Tarui's
I	II	III	IV	V	VI	VII

→ In Liver.

URONIC ACID PATHWAY (Cytosolic)

Glucose

↓ GK

Glu-6P

↓ PGM

Glu-1P

↓ UDP-Glu Pyrophosphorylase

UDP glucose

↓

UDP-Glucuronic acid → Utilized as a conjugating agent
- MPS synthesis

↓

L-Gulonate & L-Gulonolactone oxidase → abs in human

↓ L-Xylulose ↓ Vit.C [Not in human]

QQ Block in essential Pentosuria ← Xylitol dehydrogenase [X-D] —⊖→ ~~Essential Pentosuria~~

↓ L-Xylulose Reductase — ↓ L-Xylulose in urine

Xylitol

↓ D-xylulose Reductase [X-R]

D-Xylulose ⟶ PP Pathway

Heterogenous groups of compounds

Separate Lipid from Non-lipid mixture

Date
Page 48

LIPIDS

- Compounds which are soluble in Non-polar Solvent but Insoluble in polar Solvent.
  - eg: Benzene, ether, chloroform, Acetone, Formalin
  - eg: H_2O; Alcohol; Blood plasma
- Modified Bloor's classification :⇒

Simple lipid	Compound lipid	Miscellaneous / complex
alcohol + Fatty acids	[Simple + few groups]	① Fatty acids
① Triacylglycerol (glycerol + 3FA)	① Aminolipid	② Cholesterol
② ceramide / wax (sphingosine + Fatty acid)	② Sulfolipid	③ Bile acids
↓ Aminoalcohol having double bond	③ Glycolipid	④ Steroid hormone
	④ Lipoprotein	
	⑤ Phospholipid	

* Fatty acid is precursor of cholesterol.

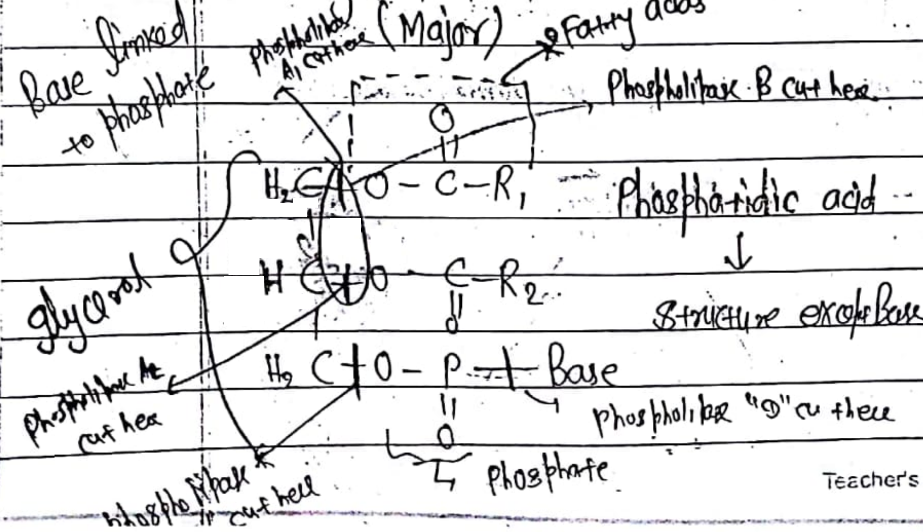
derived lipid ⇒ derived from cholesterol

both derived & precursor lipid

- No branching in it (Linear Molecule)

PHOSPHOLIPID → Plasmalogen

Glycerophospholipid (Major)



Phosphatidic acid
↓
Structure except Base

Sphingophospholipid (Rare)

↓ having choline base

eg → Sphingomyelin
↓
Sphingosine + FA + PO_4^- + Base
↓
Very huge, high M_W

Teacher's Signature ....

Barth Syndrome

→ "BARTH SYNDROME" → due to deficiency of cardiolipin.

→ "Niemann-Pick disease" → due to sphingomyelinase deficiency.

- Component of glycerophospholipid
→ glycerol + 2 FA + PO_4^- + Base

Base
- $\bar{c}$ Nitrogen
- $\bar{c}$ out Nitrogen

- Base in Sphingomyeline
↳ Choline

Sphingomyelin = ceramide + PO_4^- + choline
↓
acts as Second messenger by regulating apoptosis, cell cycle & cell differentiation.

- Depending upon the presense of Nitrogen :→

GLYCEROPHOSPHOLIPID

N_2 – containing Base

(1) Phosphatidyl Choline [Lecithin]
Most abundant phospholipid of Membrane
Choline has "labile Methyl group".

(2) Phosphatidyl ethanolamine [Cephalin] Q

(3) Phosphatidyl Serine

- Choline, ethanolamine, Serine are N_2 containing Base

Non-N_2 containing

(1) Phosphatidyl Inositol
↳ [Base → myo-inositol]
Base (Myoinositol)
Precursor of Second messenger in cells (IP_3 + DAG)
(PIP_2 $\xrightarrow[C]{\text{Phospholipase}}$ IP_3 + DAG)
DAG → "Protein kinase C" in Nucleus
IP_3 → Ca^{++} ER Release

(2) Diphosphotidyl glycerol [Cardiolipin]
↓
two molecules of phosphatidic acid held by an additional glycerol through phosphate group.
↓
- Found in inner mitochondrial Membrane. Q
- Antigenic Nature Q
- Receptor activity (play a Role in

Teacher's Signature ....

• "Glycolipids" are formed in "~~Endoplasmic Reticulum~~".

PLASMALOGEN → It is a phospholipid. [$-O-\overset{O}{\overset{\|}{C}}-R$] abt,
→ Fatty acid is not present at C_1
→ Unsaturated alcohol present at C_1 through ether linkage
→ also k/a "Phosphatidalethanolamine"

formed when a fatty acid is attached by an ether linkage at C_1 of glycerol of glycerophospholipid

→ present in - Brain Parenchymal cells } → only 10% of total
— white fibre muscle | phospholipid is plasmalogen.

eg → ① Choline ~~[illegible]~~ plasmalogen
② Ethanolamine plasmalogen.
③ Platelet activating factor

SPHINGOGLYCOLIPID / GLYCOLIPID → alcohol → always sphingosine

① cerebroside (ceramide monohexoside);
↳ Galactose > Glucose
② Globoside (ceramide oligohexoside);
globoside
↳ oligosaccharide attached to ceramide
③ Ganglioside → ceramide + glucose + galactose + NANA
ceramide + glucose + glalactose + NANA

(Sialic acid)
Most component

Simplest Ganglioside (GM3)
Simplest ganglioside
↳ Smallest
Smallest

↑ structural complexity

Tay - Sach's disease → due to deficiency of "hexosaminidase" (α-subunit)
Sandoff disease → due to deficiency of "Hexosaminidase (β-subunit)"

→ In both disease "GM_2 ganglioside" is accumulated.

→ ceramide + glucose + Galactose — N-Acetyl galactose
| NANA
⇓
GM_2

→ ceramide + glucose + galactose — N-acetyl galactose — Galactose
| NANA
⇓
GM_1 → Biggest

Ganglioside ← G M → Monosialic acid
$GM_{1/2/3}$ → is the chromatographic migration on size exclusion chromatography
↓
• Separation of any mixture into their components based on physical property of compound
eg → charge, solubility, weight, size.

GM_3 → fastest migration.
GM_1 → slowest
↓
Heavier

• Ganglioside → doesn't have phosphate

• Galactocerebroside more common than glucocerebroside
• Krabbe's disease → due to deficiency of enzyme β-galactosidase
↳ galactocerebroside accumulate in brain.

NEET16 Gaucher's disease → due to deficiency of β-glucosidase

• Extracted lipids are separated into individual class by "chromatography" NEET

Teacher's Signature

Date
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- Fatty acids liberated from adipose tissue by Lipolysis are not water soluble. attached to Albumin[Q] to be transported in Blood, or Any Plasma protein.

$\boxed{\text{FATTY ACID}}$ → Aliphatic carboxylic acid

— R COOH

Including C-atom of -COOH

- Short chain FA → 4-6 C-atom;
- Medium chain FA → 8-14 C-atom
- Long chain FA → 16-22 C-atom
- very long chain FA → > 24 C-atom

Numbering System

Delta system

$\overset{6}{CH_3} - \overset{5}{CH_2} - \overset{4}{CH_2} - \overset{3}{CH_2} - \overset{2}{CH_2} - \overset{1}{COOH}$

ω → Last carbon

ω δ γ β α → adjacent to carboxyl

Omega system

ω_1 ω_2 ω_3 ω_4 ω_5 ω_6

Last C

* "ω" family denotes the position of 1st double bond from "ω" end.

Position of double bond in FA chain

⇓

1st double bond from ω end

eg → C–C–C=C–C–C

ω_3

↓

ω_3 fatty acid

Chain Name → 6:1 ($\Delta 3$)

No. of "C"

No. of double bond

Position of double bond acc to delta system

double bond

eg → $CH_3 - CH = CH = CH - CH_2 - COOH$

ω_3 family

Chain Name → 6:2 (Δ_3 & Δ_4)

Teacher's Signature ....

• Most active Plasma lipids → Free fatty acids.

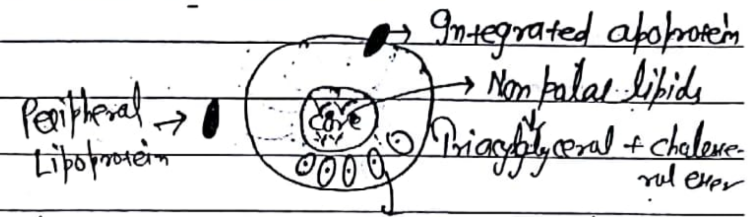
↳ Metabolized in Plasma,

LIPOPROTEINS

Lipid + Proteins (Remnants particle)

→ five types of lipoprotein → LDL, HDL, VLDL, chylomicron, & free fatty acid–albumin complex

→ Structure →

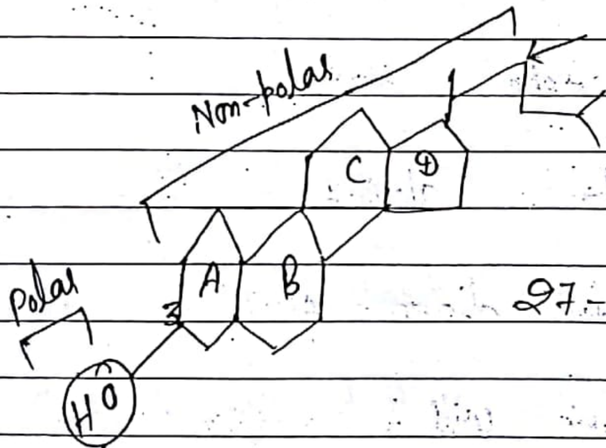


Both polar & Non-polar ← Amphipathic shell

↓

Phospholipid & free cholesterol

→ Free cholesterol →



27-carbon compound c̄ 4 rings..

* Polar Surface towards surface & Rest towards core.

↳ Makes it amphipathic

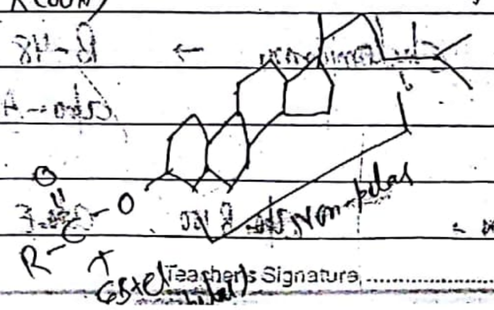
↳ attached on C_3

Free cholesterol + FA (RCOOH) ——→ esterified cholesterol ↱ Non-polar

Cholesterol Ester

↓

FA + cholesterol



Teacher's Signature ....

CETP ⇒ cholesterol ester transfer protein.

Date 24/2
Page 54

- Protein of lipoprotein is k/a "Apolipoprotein/ Apoprotein".

→ Structural component of lipoprotein.
→ Enzyme cofactor – Apo CII & Apo CI $\xrightarrow{+}$ Lipoprotein lipase
Apo A-1 $\xrightarrow{+}$ LCAT*
→ Enzyme inhibitor :- AII & CIII $\xrightarrow{-}$ Lipoprotein lipase
Apo CI $\xrightarrow{-}$ CETP*

Lecithin cholesterol acyl transferase

→ helps in recognition of lipoprotein by their Receptors.

Ligand for lipoprotein Receptors :-

- LDL receptor = apoB_{100} & apoE
- LRP = apo E
  ↳ LDL Receptor related Protein
- Remnant Receptor = Apo E
- HDL Receptor = apo A-1
- Neurodegenerative disorder = Apo D.
- apoE lipoprotein will be identified by LDL, LRP, Remnant Receptor
- LDL particle has only 1 apoprotein → apo B-100

Ligand of LDL

- Chylomicrons → B-48
  apo-A

Q: Which Receptor is prt. on the liver for LDL uptake

Ans → apo-B 100, apo E

Teacher's Signature ....

→ Chylomicrons are synthesized in intestine & transport exogenous triacylglycerol to various tissue.

	Site of Synthesis	Apoprotein
	Liver & Intestine →	A-I, A-II
	Liver	→ A-II, B100, C, E
	Intestine	→ A-IV, B-48
	Spleen, brain, Testis, Adrenal →	D

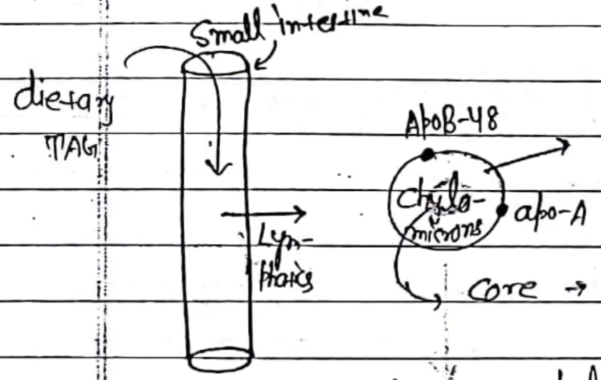
Q. Ligand of LDL :-
1. apo-B100
2. Apo-E
3. Apo-48
4. Apo A_{II}

"Serum appeared "Milky white" in ↑es chylomicrons."

CHYLOMICRON METABOLISM

Post prandial → Chylomicrons ↑ in Blood
↓
Comes from GIT mucosal cell
↓
via lymphatics enters blood.

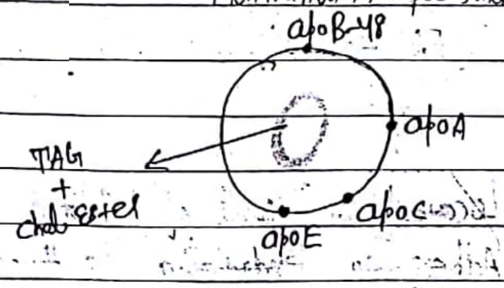
* chylomicrons :→ Principal form in which dietary lipids (exogenous lipids) are carried from Intestine to Liver



Shell → Phospholipid + Free cholesterol
(exogenous → dietary)
Core → TAG + cholesterol ester [dietary]

Nascent chylomicrons (Non-functional)
apo C
apo E (from HDL)

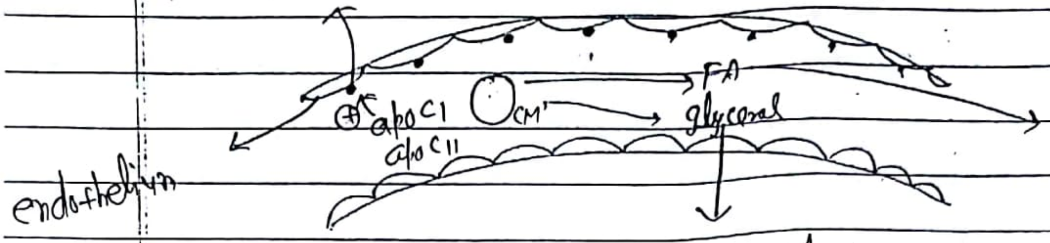
Maturation in plasma



Mature chylomicrons

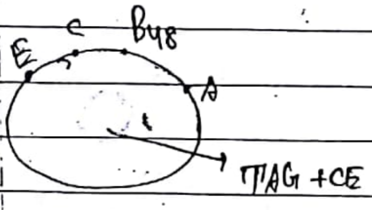
(+)nt on wall of the capillary endothelium
acts on TAG of CM core

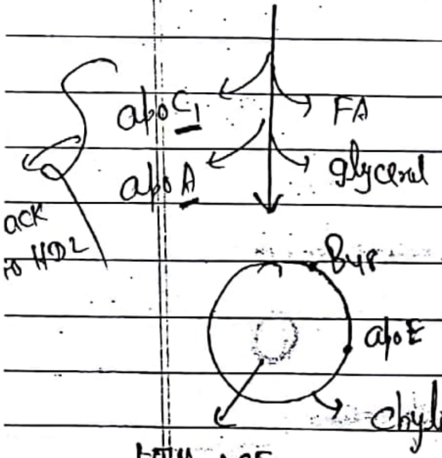
Lipoprotein lipase : enters capillary lumen



Remains in Blood
↓
goes to liver finally
↓
formed glucose

Receives fatty acids





→ Size of chylomicron ↓es after this

20% TAG Reaches liver from chylomicron Remnant.

- Chylomicron Remnant is identified by apo E

*** CET-NOV16 Chylomicron distributes dietary FA to different cells & then cleared from solution

- Lipoprotein lipase → Located on wall of blood capillaries
  - Adhere to endothelium via Heparan sulphate
  - Heparin release it.

Teacher's Signature ....

VLDL → TG Rich Particles & transport endogenous TG from Liver to peripheral tissue.
LDL → Cholesterol Rich Particles & are the Major source of cholesterol to Peripheral tissue;
HDL → Transport cholesterol from Peripheral tissues back to Liver

Date
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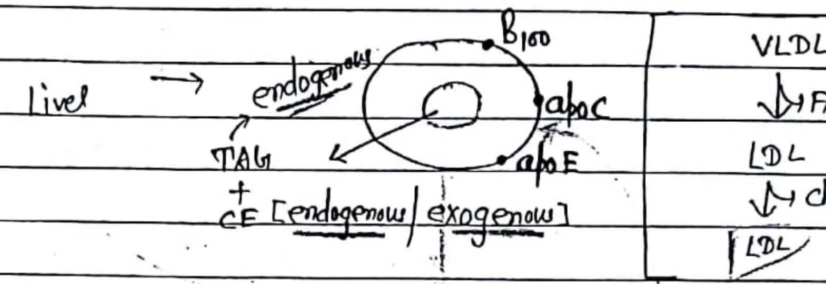
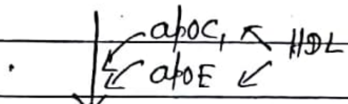
(30)

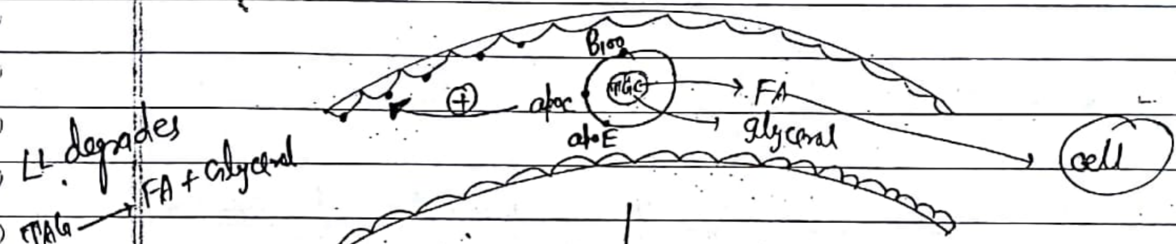
VLDL TRANSPORT → distributes FA & cholesterol endogenously to cells.

→ VLDL are produced in liver & intestine.



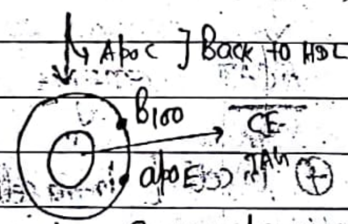
Liver form Nascent VLDL; after adding apo C_1 & apoE they form Mature VLDL.





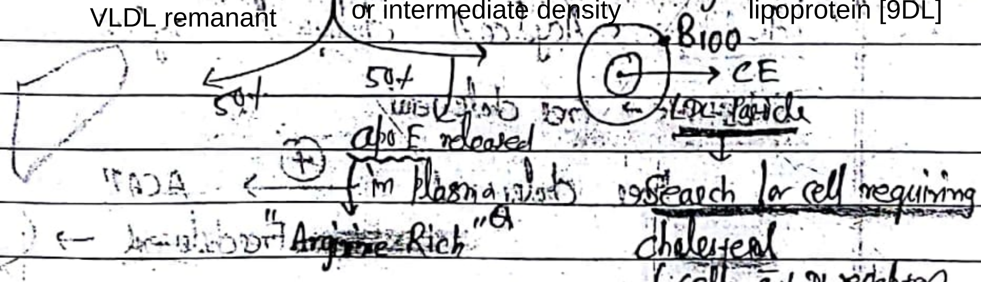
After giving FA into cells & glycerol to Blood

VLDL remains in Blood (size↓)



VLDL remanant or Intermediate density lipoprotein [IDL]

VLDL remanant or intermediate density lipoprotein [9DL]



arginine rich Teacher's Signature

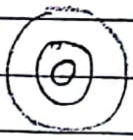
• LDL contains only one apoprotein ⇒ $ApoB_{100}$

B_{100}

LDL

Endocytosis

LDL receptor



← Extrahepatic cells

Endosome
↓
Chol. Ester
↓ Lysosomal Enzyme
converted to cholesterol
↓
Free
↓
Reaches cholesterol pool

denovo

→ Source of cholesterol in cholesterol pool –
i) LDL
ii) Denovo cholesterol synthesis.

→ LDL receptor lost during endocytosis is Resynthesized

→ If cholesterol ↑es in Pool →
- LDL Receptor Synthesis down Regulated;
- ↓ denovo synthesis;
- ACAT enzyme stimulated

↓

intracellular ACAT Involove in cholesteral

⊕ In cell [Intracellular ACAT, involved in cholesteral regulation]

→ $\boxed{ACAT}$ → Acyl CoA cholesterol acyl CoA transferase
↓
Substrate → Free cholesterol

If ↑ in free cholesterol —⊕→ ACAT
Free cholesterol → Cholesterol ester

Teacher's Signature

HDL → good cholesterol → Aerobic exercise ↑es the level of HDL
LDL → bad cholesterol

- oxidized LDL is more atherogenic b/c it accumulates in Macrophage

$\boxed{\text{LDL Receptor}}$ → prt. on hepatic & Extrahepatic cells,

→ Identify B-100 & apo E

→ Taken by Endocytosis

→ QQ Clathrin coating ⊕

AIIMS → ↑ cholesterol downregulates LDL Receptor

$\boxed{\text{HDL METABOLISM}}$

HDL → Removes extracholesterol from cell & gives it back to liver.
↳ Mostly synthesized in Liver.

⇓

Reverse cholesterol transport.

Reverse Cholesterol transport :-

Liver → ApoA1 ApoC ApoE

Discoidal HDL

LCAT
↓
Lecithin cholesterol acyl transferase

Lacuna

extrahepatic pool

Cholesterol

↑ Lecithin
Periphery → Phospholipid + cholesterol (free)
core centre → few cholesterol esters
- Apoproteins → ApoA, C, E
activates LCAT [in plasma]

Lecithin + Cholesterol Lecithin + Cholesterol

In Blood

↓ LCAT → transfers acyl from lecithin to cholesterol

Lysolecithin + Cholesterol esters

Lysalecithin

RxN is occuring in HDL shell

goes to HDL core

- a space is created in HDL shell → k/as LACUNA

Substrate → free cholesterol + phospholipid (lecithin)

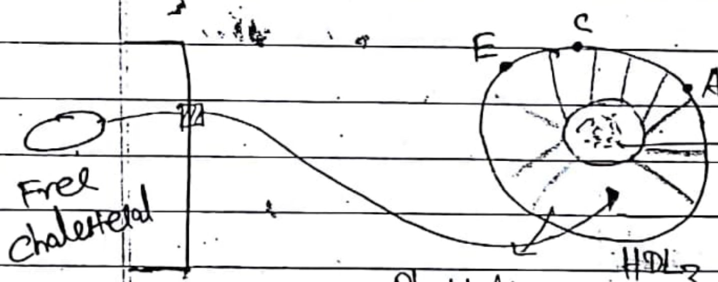
Teacher's Signature ....

→ free cholesterol comes to lacuna & due to LCAT forms cholesterol ester
↳ goes to core

→ LACUNA → accomodates the free cholesterol coming from different cells (extrahepatic cells)

↓

Now discoidal HDL will become "spherical HDL"



→ ↑ cholesterol ester

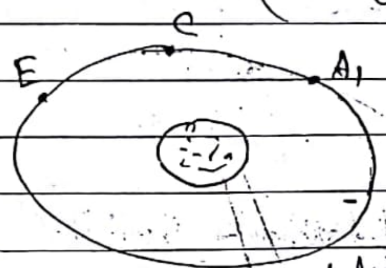
→ Lacuna
↳ extrahepatic cholesterol accomodated

Phospholipid + free cholesterol

HDL3 (spherical)

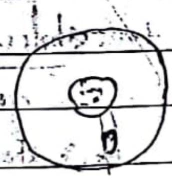
↓

HDL2 (Process is continous
↳ more chol. ester in core)
∴ Big size



if HDL2 is saturated c̄ cholesterol Ester in core, the last free cholesterol will block the lacuna as LCAT will no more act due to feed back inhibition by chol. ester

HDL2 will deliver cholesterol to liver



While going to liver, HDL_2 encounters Remnants;

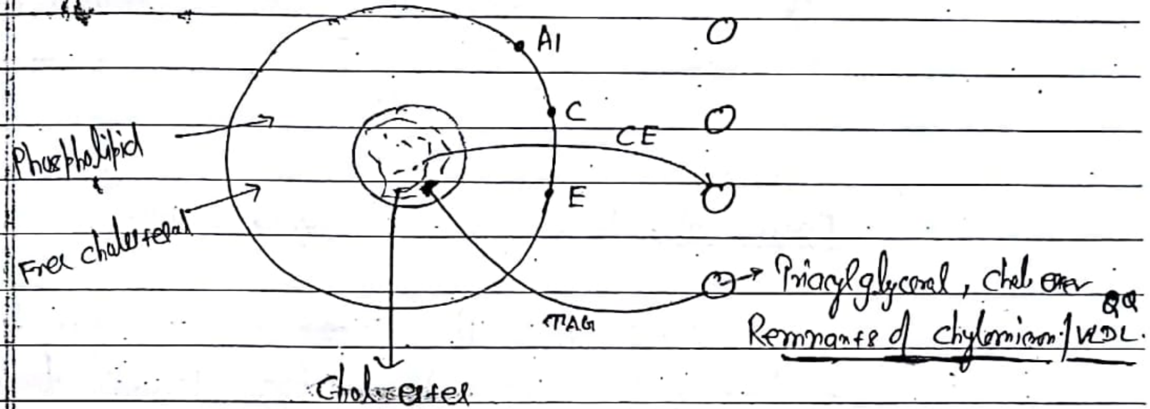
⇓

HDL gives → Some amount of cholesterol esters to Remnants & in Return Remnants transfers TAG to HDL.

⇓

Liver

This exchange of CE for TAG is mediated by CETP (Chol. Ester transfer protein) QQ CETP-JU



↓

as chol. ester ↓ in core

↓

LCAT becomes functional

↓

Lacuna created.

↓

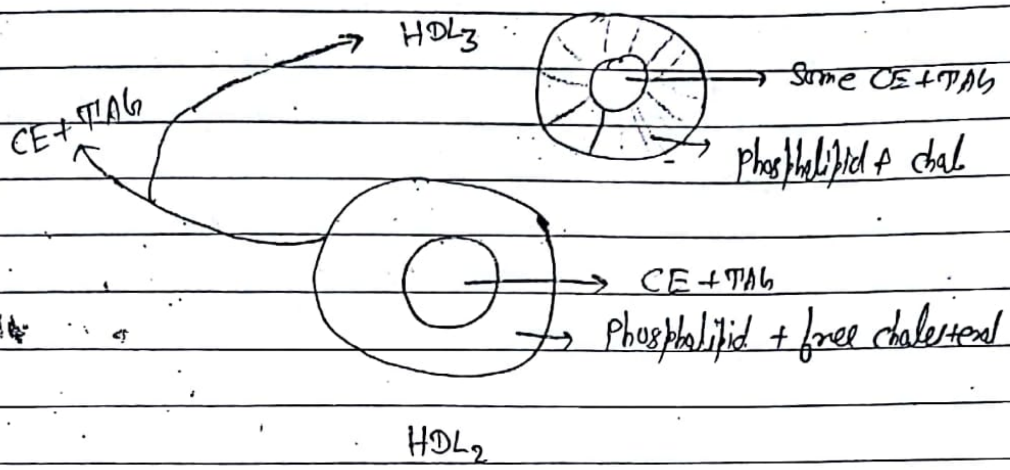
again free cholesterol comes to HDL2 from peripheral cell

again free cholesterol comes to HDL2 from peripharal cell

Function of CETP → ↓ Tes the Scavenging action of HDL
· Remnant particles helps in Reverse cholesterol transport.

Teacher's Signature ....

- In case of fast circulation no exchange is possible & HDL_2 reaches Liver.



→ Fate of HDL in Hepatocytes [Liver] :-

① Some amount of CE & TAG is taken by liver & Remaining HDL is send back to circulation in a Smaller form k/as "HDL_3".

↓

converts to HDL_2 in plasma

↓

again it will reach liver

$HDL_2 \longrightarrow HDL_3$ in liver Surface
HDL2 HDL3 in liver surface
} HDL cycle
$HDL_3 \longrightarrow HDL_2$ in plasma
HDL3 HDL2 in plasma

② Each & Every component of HDL will be tackled by hepatocytes except :- ApoA1

↓

escape to circulation

filtered in Urine (Max^m)

or

ApoA1 become a component of PreP HDL
PreP HDL

↓

Teacher's Signature ... formed in plasma
Formed in plasma

Date
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(33)

- Dietary cholesterol is transported from Intestine to Liver → By chylomicron - chylomicron Remnant system,
- From Liver cholesterol is incorporated into VLDL & is distributed to extrahepatic tissue through LDL

HDL transport cholesterol from extrahepatic tissues to liver

Pre β HDL → empty core
→ Shell → Phospholipid + free cholesterol
- Apo A_1

[Flat, small structure]
- Apo C & E may be borrowed from mature HDL [2,3]
- very Rare form of HDL

- due to presence of Apo A_1 → LCAT activity is also present

↓

∴ Cholesterol is again converted to chol. ester

↓

Lacuna formed

↓

little large in size → finally discoidal HDL is formed

↓

Reverse cholesterol transport continues

Only loss of HDL is loss of A_1 in the urine.

HDL c̄ max^m Scavenging capacity → Pre β HDL
↓
core empty

Source of discoidal HDL → Liver, GIT, Pre β HDL

→ Transporters of cholesterol on extrahepatic cells →
- ABC-A1 [ATP Binding Cassette]
- ABC-G1
- SR-B1 [Scavenger receptors]

give cholesterol to HDL_3 & discoidal HDL.

Teacher's Signature ....

Apo E → E_1 → Good ligand action
→ E_2 → Bad → Not easily recognized by Receptor
→ E_3 → Good

HDL → α-Lipoprotein

- SR-B1 plays double role in HDL metabolism
  ↳ (+)nt on Extrahepatic & hepatic cell

function is different on both cells. | Extrahepatic: gives free chol. from cell to HDL | Hepatic: gives chol. ester from HDL to hepatic cell

Q Max^m TG → Chylomicrons
NEET!! Max^m exogenous TG → "
Max^m endogenous TG → VLDL
Max^m cholesterol → LDL
Max^m Lipid content, Min^m protein content → Chylomicrons

Min^m lipid content & Max^m protein content → HDL.
* Largest Lipoprotein ⇒ Chylomicrons
* Least density ⇒ Chylomicrons

HYPERLIPOPROTEINEMIAS

- also k/as "hyperlipidemias or dyslipidemias"
- Elevation of one or more lipoprotein in the plasma
- Fredericksons classification ↳

Type	Primary disease	Basic defect	Sr. Cholesterol	Sr. Triglyceride	Elevated Lipoprotein
I	Familial Lipoprotein lipase deficiency	LPL deficiency	N	—	Chylomicrons VLDL
IIa	Familial Hypercholesterolemia	LDL-receptor Mutation/ Apo B-100 Mutation		N	LDL
IIb	IIa + Hyper-secretion of VLDL	Overproduction of apo-B	—		

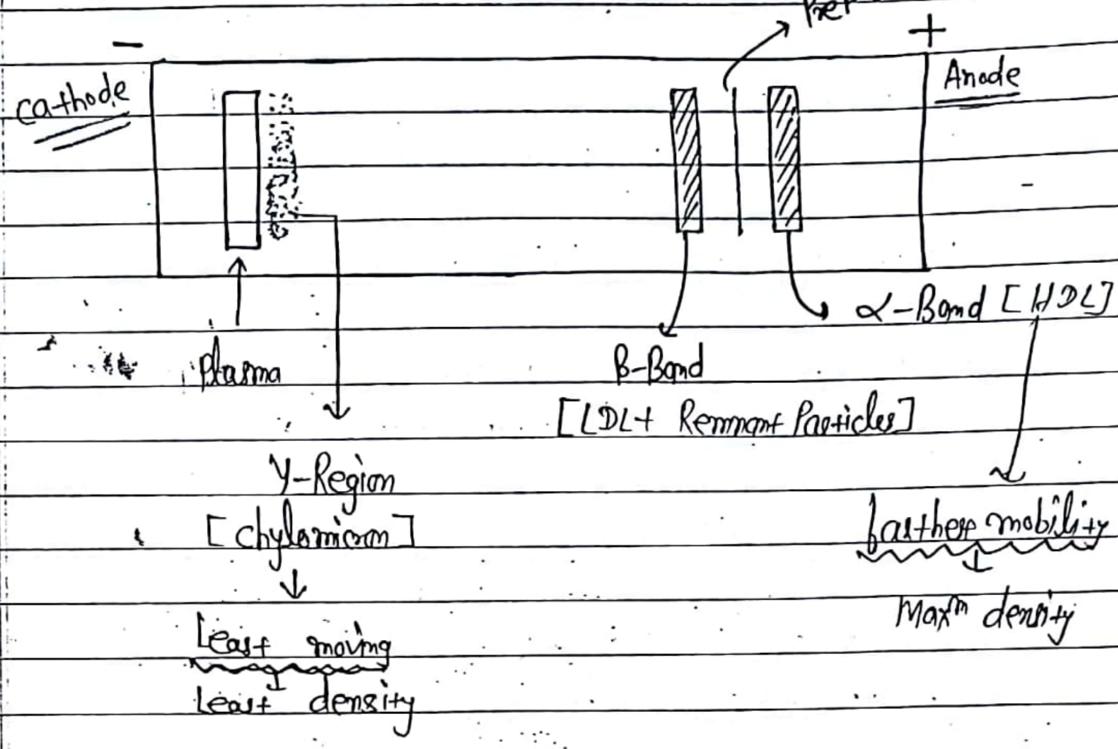
Type	Primary disease	Basic defect	Sr. cholesterol	Sr Triglyceride	Elevated lipoprotein
III	Familial dys-β lipoproteinemia / Broad β disease / Remnant Removal disease	Abnormal Apo E turn into (Apo E2) → Not easily detectable	↑	↑	VLDL Remnant CM Remnant (IDL)
IV	Familial hypertri-glyceridemia	Not known [a/w hyperinsulinemia]; develop chronic Pancreatitis	N	↑↑	VLDL
V	F combined hyperlipoproteinemia	deficiency of apo C	N	—	CM & VLDL
	F hyper α lipoproteinemia hyper ↳ only ✓ Lipoproteinemia ; which are good for health.				↑ HDL
	Hepatic lipase deficiency				HDL & TG rich VLDL remnant
	Familial LCAT deficiency				HDL remains as Nascent discoidal
	Familial Lipoprotein (a) excess			—	Premature atherosclerosis

• LDL is Smaller than VLDL.

* Density of Lipoproteins in decreasing order → HDL > LDL > IDL > VLDL > Chylomicrons
PGCC 66

NEET '16

LIPOPROTEIN ELECTROPHORESIS



Criteria of motility → Charge : Mass Ratio

Highest Ratio → Highest mobility

HDL → α-Lipoprotein
LDL → β-Lipoprotein
VLDL → Pre-β Lipoprotein

• Lipoprotein (a) → (N) → 5 mg/dl
→ if level ↑ → Premature atherosclerosis.
↳ it is LDL particle c̄ Apo(a)
↓
Similar to plasminogen

Teacher's Signature ....

Lipoprotein	Source of origin
Chylomicrons →	Intestine
Chylomicrons Remnants →	Chylomicrons
VLDL →	Liver
IDL, LDL →	VLDL

Plasminogen —TPA→ Plasmin
↓
Fibrinolysis

APO(a) ⊖ TPA
↓
if apo(a) also present
c̄ plasminogen
↳ Level of plasmin ↓ → Fibrinolysis ↓
↓
Small clots accumulate
↓
Atherosclerosis state.

HYPOLIPOPROTEINEMIA

NEET'16 ① AB lipoproteinemia → ~~Rare disorder due to defect in synthesis of apo B protein.~~
Caused by defect of Microsomal Triglyceride transfer protein NEET'16
↓
K/as "Bassen-Kornzweig Sy."

- Fat absorption is affected & → No CM/VLDL/LDL
the affected child presents with Steatorrhea. HDL → N ; only α-band seen; there is No β-Band NEET'16
- All fat soluble vitamins (A, D, E, K) affected. Vit. E deficiency presents as Retinitis Pigmentosa & Subacute combined degeneration.

② Familial alpha-lipoprotein deficiency → Total or Near-total absence of HDL
↓
K/as "Tangier disease" } → Caused by ATP Binding cassette transporter I (ABC-I)
Fish eye disease
Apo A1 deficiency

Caused by Partial Lecithin cholesterol Acyl transferase (LCAT).
- Complete LCAT defect cause "Hemolytic Anemia & Renal failure".

Teacher's Signature ....

Date
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FATTY ACID OXIDATION

β-oxidation	α-oxidation	ω-oxidation
↓	↓	↓
Lots of ATP Produced	No ATP	No ATP
↓	↓	↓
Acetyl CoA produced	CO_2 produced	"ω-carbon" CH_3 converted to COOH
		↓
		Rarely seen in Brain Parenchymal cell & white fibre muscle
		↓
		Dicarboxylic acid produced
		↓
		Seen for medium chain fatty acid oxidation
		↓
		Max^m steps occur in Endoplasmic Reticulum.

ω γ β α
$CH_3 - CH_2 - CH_2 - CH_2 - COOH$
↳ Last carbon ↓
α-oxidation

α-OXIDATION

→ Occurs in "Peroxisome"

→ For oxidation of Branched chain fatty acids
↓
eg → Phytanic acid

→ Refsum's disease → accumulation of phytanic acid
↓
defect of α oxidation which impaired β-oxidation of Phytanic acid.

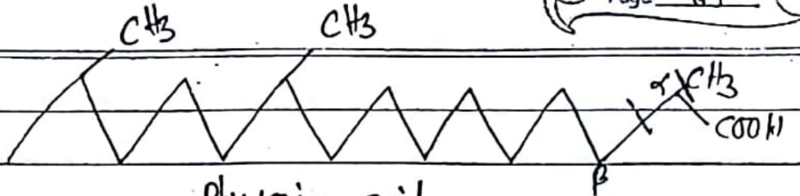
Neurological disorder

→ Source of Phytanic acid → Dairy Products, cereals, Plant food
Source ov Phytanic acid Dairy products; cereals, plant food

→ Phytanic acid → high Mw, Branched chain fatty acids
Phytonic acid high Mw, Branched chain fatty acids

Teacher's Signature ....

Stearic Acid ⇒ 18-C



Phytanic acid

→ Major oxidation here also → β-oxidation
but for removal of CH_3 on α-C, → α-oxidation occurs.

→ ATP is produced during Phytanic acid oxidation

→ Refsum's disease is due to defective α-oxidation enzyme [Phytanic acid α-hydroxylase] OR Phytanic acid α-oxidase, which impairs β-oxidation to proceed.

Brain & RBC doesn't utilize Fatty acid ← $\boxed{\text{β-OXIDATION}}$ → Mitochondria (majority) / → Peroxisomes

β-oxidation occurs in DM & Starvation.

↓

∴ lack of effective Insulin

↓

So, β-oxidation occurs

[Insulin prevents β-oxidation]

Prevents

TAG	Adipose cell

↓ Hormone sensitive lipase → active in phosphorylated form (⊕ in adipocytes)

↑⊕ • Glucagon • Epinephrine

Fatty acid (in Blood)

└ Long-chain fatty acid (eg. Palmitic acid)

↓ enter diff cells il ≥ 10mmol

Site of β-oxidation

Mitochondria

Mitochondrial Matrix

↓ Fatty acid → Liver

CELL

• Activated form of FA

↓

Acyl CoA

- Synthetase – ATP req. → class VI enzyme
- Synthase – ± ATP req. → Any class.

- FA is not permeable to outer or inner mito. membrane

↓

So, shuttle sys. required

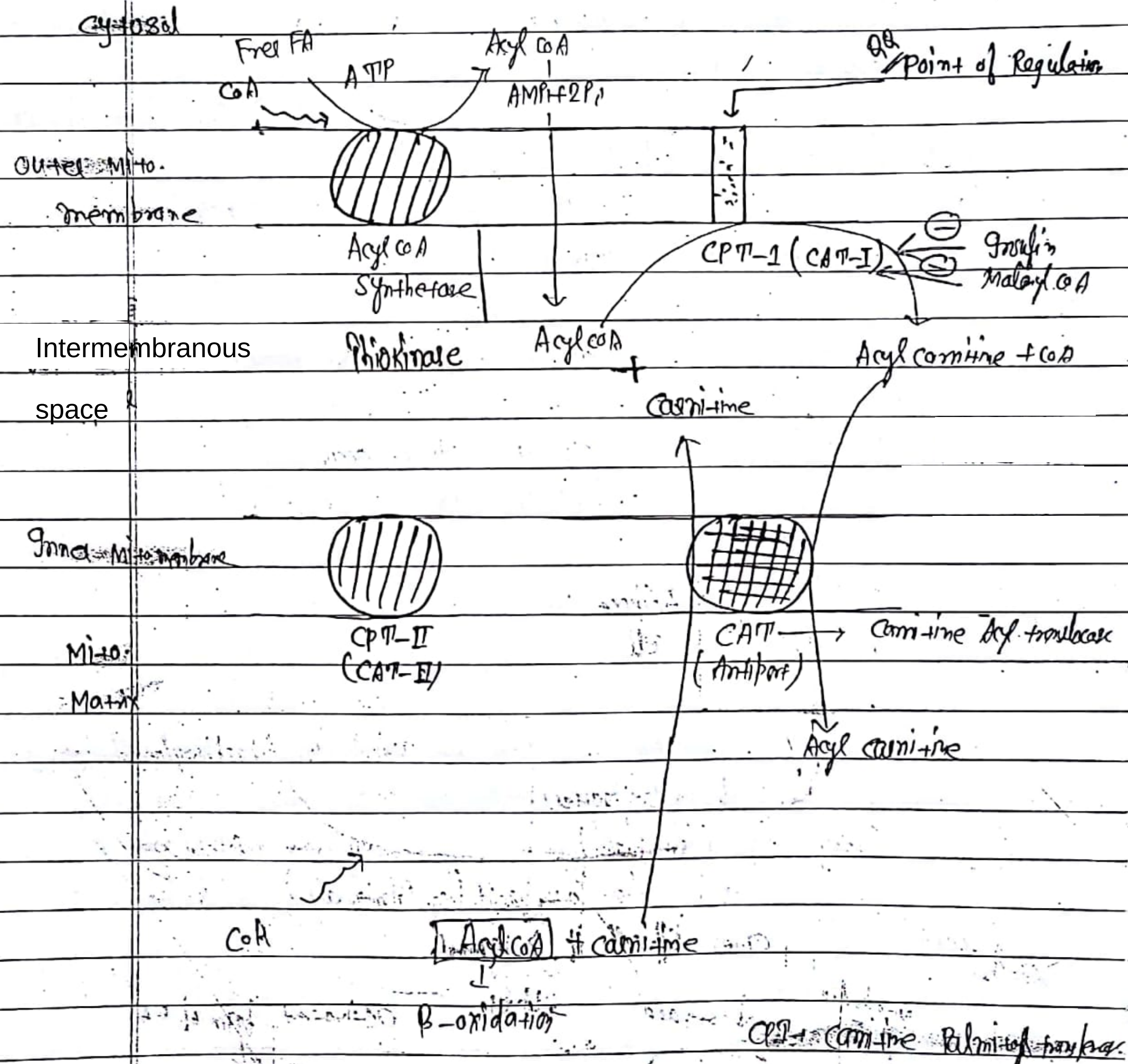
↓

Carnitine Shuttle

↓

transfer FA to mitochondrial matrix

CARNITINE SHUTTLE



- In infants carnitine is not present, so, β-oxidation is affected – Baby may become hypoglycemic

→ • Point of Regulation → CPT–I

• Insulin inhibits (Blocks) → CPT–I
due to formation of malonyl CoA → Block CPT–I

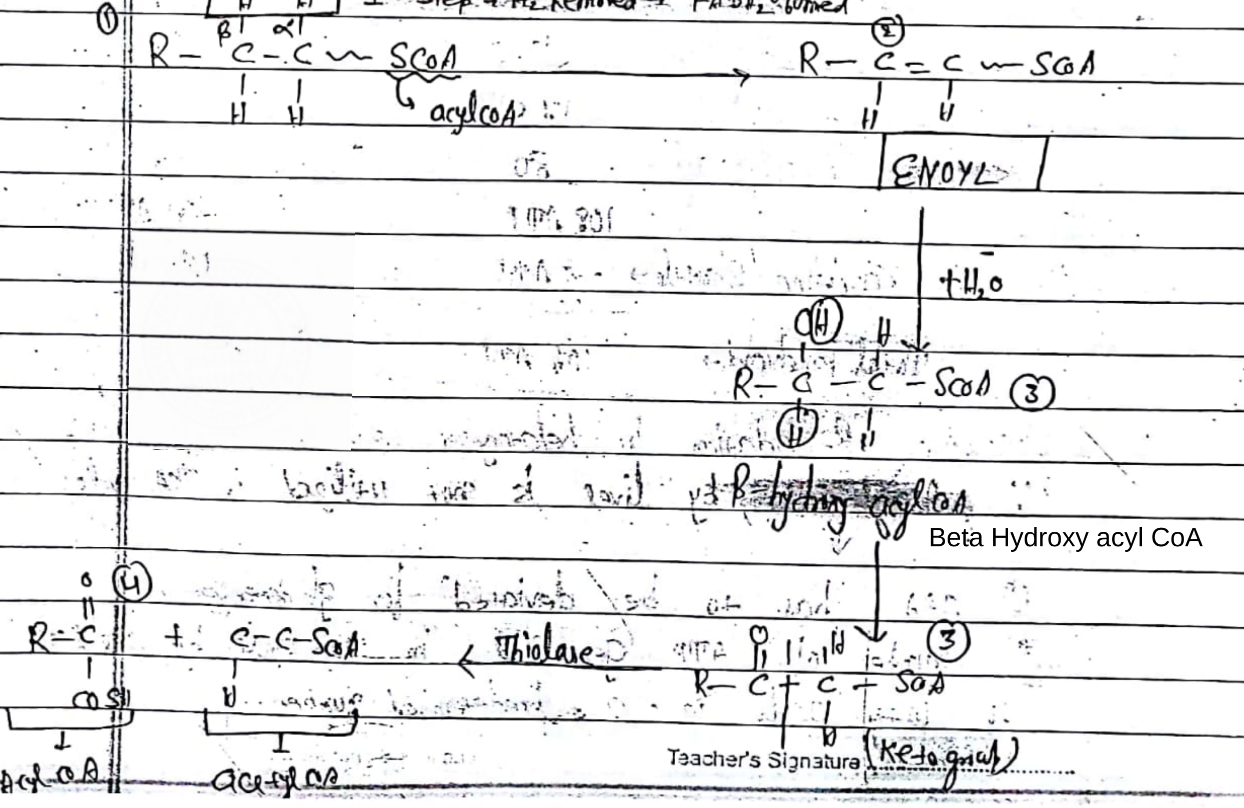
→ FA → if 16C → 7 cycles of β-oxidation

Acyl Co-A (16C)

1st cycle of β-oxidation
- Acyl CoA dehydrogenase [$FADH_2$] + coenzyme → FAD (remove H⁺)
- Hydratase (Enoyl CoA Hydratase enzyme)
- β-hydroxy acyl co-A dehydrogenase [NADH] → coenzyme – NAD
- Thiolase

Acyl Co-A (14C) + Acetyl Co-A (2C)

1st step → H_2 Removed → $FADH_2$ formed



Teacher's Signature

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Acyl CoA (14C) + Acetyl CoA (2C)

↳ $FADH_2$
↳ NADH
↩ H_2O

Acyl CoA (12C) + Acetyl CoA (2C)

Acetoacetyl CoA (4C)

2 Acetyl CoA

$\boxed{\text{No. of cycles} = \frac{\text{No. of C} - 2}{2}}$

if 16C & zero double bond = 7 $FADH_2$; 7 NADH & 8 Acetyl CoA
if 16C & x No. of double bond : Reduce x from 7 $FADH_2$.

eg → 20C → $\frac{20-2}{2}$ = 9 cycles

		New ATP	Old calculation
16C → 7 cycles			
	7 $FADH_2$	10.5	14
	7 NADH	17.5	21
	8 Acetyl CoA	80	96
		108 ATP	131 ATP
	Carnitine Shuttle →	−2 ATP	2 ATP
	Total produced →	106 ATP	129 ATP

Excessive β-oxidation in hepatocytes will result in ketogenesis →
∵ Acetyl CoA by liver is not utilized in TCA cycle;

① OAA has to be deviated for gluconeogenesis
② Number of ATP generated in TCA cycle by acetyl CoA of fatty acids is a predetermined number

Teacher's Signature ....

*

Ketogenesis

Liver mitochondria Matrix

Thiolase
↓
Reversible enzyme

Acyl CoA
↓
(4C) Acetoacetyl CoA
↕ Thiolase
Acetyl CoA ↑↑

if acetyl CoA ↑↑
↓
then acetoacetyl CoA forms

Acetoacetyl CoA + Acetyl CoA $\xrightarrow{\text{HMG CoA Synthase}}$ HMG CoA (6C)

Rate limiting enzyme

HMG CoA (6C)
↓ HMG CoA Lyase
Acetoacetate (4C) + acetyl CoA
↑
1st Ketone body generated
[Precursor of other KB]

Acetoacetate
→ β hydroxy butyrate dehydrogenase → β-hydroxybutyrate
→ CO_2 → Acetone

Acetone
[irreversible & spontaneous process]
↓
No enzyme req.
↓
Volatile gas
↓
Exhaled, So, not present in plasma

β-hydroxybutyrate : Acetoacetate = 10 : 1
↓
Predominant Ketone bodies in Ketosis
↓
Lack of insulin is req.
eg. DM & starvation

Utilization & synthesis of kB its require Mitrochondria

Synthesis of FA -----------------> Cytoplasm

Utilization of FA ----------------------> Mitrochondria

→ Insulin inhibits both → β-oxidation, Ketogenesis

Insulin inhibits boths

Teacher's Signature ....

* - Minm 48 hr of Starvation Required if Ketogenesis is to takes place

Utilization of KBs :- in peripheral cell

↓

all cells can use except → RBC & liver

$\text{Acetoacetyl CoA (4C)} \xrightarrow{\beta\text{-oxidation}} 2\ \text{Acetyl CoA} \rightarrow \text{TCA cycle}$

Acetoacetyl CoA (4C)

↑ CoA Transferases [Thiophorase]

Succinyl CoA → Succinate

Acetoacetate

↕ β-OH butyrate dehydrogenase

β-OH butyrate

→ • Liver lacks Thiophorase enzyme } So, can't utilize
• RBC → No mitochondria } ketone bodies

So, can't utilize ketone bodies

→ only fuel for RBC → for energy → glucose

⇓

Lack of mitochondria ∴ can't utilize KB & FA

→ FA can't utilized by → Brain & RBC

• Common enzyme for Ketogenesis & Ketolysis

⇓

β hydroxy Butyrate dehydrogenase

Teacher's Signature ....

Beta Hydroxy Butyrate dehydrogenase

* Short chain Fatty Acid = 4-6 carbon
* Medium chain Fatty Acid = 8-14 carbon
* Long chain Fatty Acid = 16-22 carbon
* Very Long chain Fatty acid = ≥ 24 carbon

chain length (≥ 24 carbon)

Peroxisomal β-oxidation

- Seen only for ~~very long chain FA β-oxidation~~ very long chain FA Beta oxidation
  ↓
  Can't enter mito. matrix
- Similar cycle as that of ~~mito. β-ox occurs~~ in peroxisomes; occurs
  but complete oxidation is not taking place in peroxisomes.

End product → Octanoyl CoA (8C)
↓
~~Will reach mito. matrix~~
↓
~~Complete β-oxidation occurs here.~~

AIIMS NOV'17
Less ATP production in each cycle as compare to Mitochondrial β oxidⁿ.

Zellweger Syndrome / cerebro hepato Renal syndrome
↓
↳ Also accumulated in Mucle.
~~very long chain fatty acid are accumulated.~~

→ No. of peroxisomes are ~~Nil or Normal~~
↓
~~without enzyme~~ without enzyme
i.e. ~~Empty peroxisome~~ empty peroxisome
~~or ghost peroxisome~~ or ghost peroxisome

b/c of FADH₂ ↑ → O_2^- → SOD → $\boxed{H_2O_2}$

Teacher's Signature ....

Liver & Lactating Mammary glands are the main organs for lipogenesis.

Fatty Acid Biosynthesis → ~~cytosolic~~ * Cytosalic

- Precursor → Acetyl CoA from glucose / Fructose
  (+) in mitochondria

- Occurs in Hepatic & Extrahepatic cells.

Mitochondria | Cytosol

Acetyl CoA

OAA $\xrightarrow{\text{Citrate Synthase}}$ Citrate → Citrate

Citrate $\xrightarrow{\text{ATP, Citrate lyase}}$ OAA (4C) + Acetyl CoA (2C)

OAA (4C) $\xrightarrow{\text{Malate DH}}$ Malate (4C) [NADH → NAD^+]

Acetyl CoA (2C) $\xrightarrow{\text{Acetyl CoA Carboxylase OR Malonyl CoA synthetase}}$ Malonyl CoA (3C)

Malate (4C) $\xrightarrow{\text{Malic enzyme [MDH]}}$ Pyruvate (3C) [$NADP^+$ → NADPH; → CO_2]

- Malic enzyme is a Source of NADPH

→ Rate limiting enzyme of FA synthesis

NEET'16

Acetyl CoA $\xrightarrow[\text{Malonyl CoA synthetase}]{\text{Acetyl CoA carboxylase OR}}$ Malonyl CoA

(+) Insulin, Citrate — Both needed together

Mn^{2+}, CO_2, ATP

(Biotin Req)

∞ ∞
∞ ∞
Inactive enzyme (Dimer)

Citrate → (+ve allosteric Modifier)

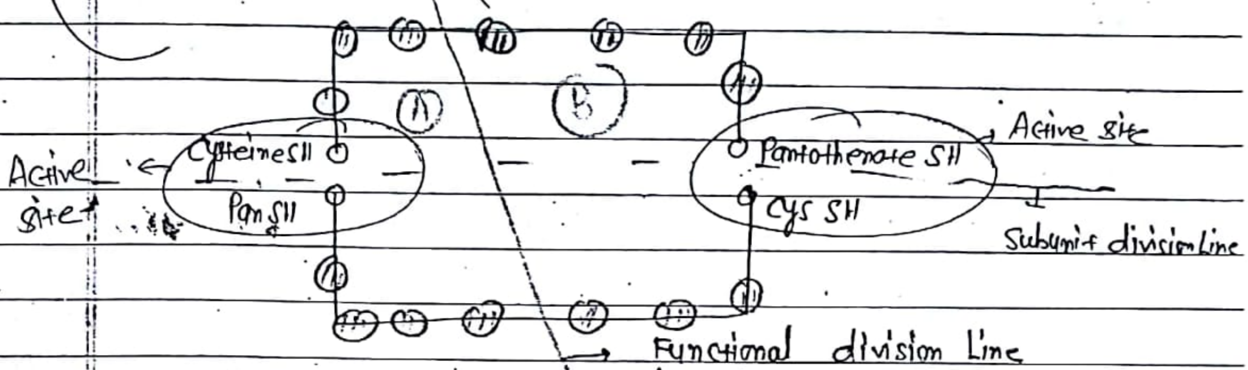
→ 26-30 Units

oooooooo
Active enzyme (Polymer)

each unit should be dephosphorylated

* There is No loss of enzyme Unit in this arrangement ; b/c there is 2 Product formed @ different sites.

Malonyl CoA
↓
Reaches FAS complex [⊕ in cytoplasm] (Fatty acid Synthase complex)
(Dimer)



→ 2 enzyme of FAS complex present in Antiparallel fashion.

→ 2 cycles are going together on FAS → due to presence of 2 active sites.

→ Never all the catalytic domains of one Polypeptide is used for catalytic activity @ one active site. Rather part of one enzyme unit & part of Another enzyme unit are used for activity @ one active site.

→ In each unit of FAS → ~~1 catalytic activity ⊕~~

PanSH — ① — CysSH ← Acetyl CoA (2C) (Acetyl transacylase)

CysSH — ② — PanSH ← Malonyl CoA (3C) (Malonyl transacylase)

Condensation + Acetyl CoA (2C)

↓ Ketoacyl synthase

PanSH — ① — CysSH

CO_2

CysSH — ② — PanSH — (4C) Ketoacyl CoA

↓ Remove keto group

→ 1st cyclical Rxn differs from subsequent cyclical Rxn :- ① Two Precursor are Required (Acetyl CoA & Malonyl CoA) but subsequently only one precursor Required (Malonyl CoA)

② Gain of 1st cycle is 4-C Acyl chain, but subsequently only two carbon is added on elongating FA chain

Elongation of Pre-existing Fatty Acids →

(A) Microsomal (Endoplasmic Reticulum) :- elongation of Palmitic acid into Longer chain FAs.

(B) Mitochondrial :- elongation of short & Medium chain fatty acids containing less than 16-Carbon atoms

Date ______ Page 78

occurs in "Anaerobic cond^n".

Favoured by high $NADH/NAD^+$ ratio in cells.

by help of enzyme Elongase & Desaturase

PanSH —— (1) —— Cys SH

Cys SH —— (2) —— PanSH — acyl CoA (4C)

} Keto Removed

PanSH —— (1) —— Cys SH — acyl CoA (4C) ← New cycle

↓

Malonyl CoA come

↓

Cys SH —— (2) —— PanSH — Malonyl CoA (3C) displaces acyl CoA to cys-SH

↓

Keto acyl synthase - adds acetyl CoA

↓

Pan-SH —— (1) —— cys SH

↳ CO_2 *

Cys SH —— (2) —— PanSH — Ketoacyl CoA (6C)

→ 1st cycle of FA synthesis differs slightly from subsequent cycles, in the sense in first cycle we need two precursor molecules

a) Acetyl CoA

b) Malonyl CoA

but subsequently we need only one precursor molecule which is malonyl CoA *

Mnemonic

PM
AC

Prime Ministers travel in AC

PanSH ← PM → Malonyl CoA

acetyl CoA ← AC → cys SH

Teacher's Signature ....

→ We get the final product at Pan SH.

→ Mammalian cell can only form "Palmitoyl CoA (16C chain)"

→ Fatty acid Synthase enzymes

① Acetyl transacylase; } Part of enzyme MAT — Malonyl acetyl transferase
② Malonyl transacylase;

③ Ketoacyl Synthase; (Involved in condensation) ↳ decarboxylating enzyme

Removes the Keto group.

④ Ketoacyl Reductase (Uses NADPH to convert Keto to Hydroxy)

⑤ Hydratase enzyme (Remove water) dehydration

⑥ Enoyl Reductase (Need NADPH & add H_2 to remove double bond

⇓

We get Acyl CoA (4C)

⑦ Thioesterase ↳ to remove the final product [Palmitoyl CoA 16C] from Pantothenate site

Processive Enzymes

→ Those enzyme complexes which are involved in cyclical reaction and where the gain of previous cycle is not dissociated from the active site and enzyme complex along with the gain of previous cycle as such is going to enter in subsequent cycle

Teacher's Signature ....

Like Insulin; Somatomedin (Insulin-like growth factor) has antilipolytic effect.

Eg. → FAS complex;
Glycogen Synthase;
DNA Polymerase;
RNA Polymerase;
Peptidyl transferase

FATTY LIVER

- Factors Causing Fatty liver :- Puromycin
  Ethionine
  CCl_4
  Chloroform
  Phosphorus
  Lead
  Arsenic
  Orotic acid
  Protein deficiency
  Vitamin deficiency
  EFA deficiency
  Alcohol.

- Lipotropic factors → Substance the deficiency of which (prevent fatty liver) Cause fat (Triacylglycerol) to accumulate in liver.

  Eg → Choline;
  Lecithine
  Methionines
  Vit E;
  Selenium;
  W_3 fatty acids
  Vit B_{12};
  Folic acid;
  Inositol
  betaine.

Teacher's Signature ....

Thiolase — βox } Mito
— Ketogenesis }
— Chol. synthesis → cytoplasm

NEET Progesterone (Steroid Hormone) Requires HDL to synthesized

" Mg^{+2} " Require QA

CHOLESTEROL SYNTHESIS → Cytosolic

- Acetyl CoA (from glucose, Fatty acids)
  ↳ Mitochondrial

* Double bond in cholesterol is found in "Ring B"

(Mito) | (cytosol)

Acetyl CoA + OAA → Citrate → (citrate transporter | Tricarboxylate transporter) → Citrate

Citrate —ATP citrate Lyase→ OAA + Acetyl CoA (2C)

Acetyl CoA (2C) + Acetyl CoA (2C) —Thiolase→ Acetoacetyl CoA (4C)

Acetoacetyl CoA (4C) + Acetyl CoA —HMG CoA Synthase→ HMG CoA (6C)

HMG CoA (6C) — Activated in Dephosphorylated form

HMG CoA (6C) —HMG CoA Reductase (↳ Rate limiting enzyme); NADPH → $NADP^+$→ Mevalonate (6C)

Mevalonate (6C) → CO_2 → Isoprenoid (5C)

Isoprenoid (5C) × 2 → Geranyl Pyrophosphate (10C)

Geranyl Pyrophosphate (10C) + Isoprenoid → Farnesyl Pyrophosphate (15C)

Farnesyl Pyrophosphate (15C) × 2 → Squalene (30C)

Squalene (30C) → Lanosterol (30C)
↳ 1st compound which gives ringed structure of cholesterol

Lanosterol (30C) → Zymosterol (27C) → Desmosterol (27C) → Cholesterol (27C)

Teacher's Signature

- Cholesterol is the imp. precursor for synthesis of → Steroid hormones; Vit. D[Q]; Bile acids[Q]; Progesterone, estrogen etc.

NEET PG '16 Products of HMG CoA :⇒
- Cholesterol;
- Heme A
- Bile Acids;
- Prenylated protein
- Ubiquinone;
- Dolichol;
- Ketone bodies

HMG CoA Reductase → active in dephosphorylated form

(+) ↑ Insulin, Thyroid

(−) ↑ Glucagon, Glucocorticoids, Statins, Bile acids, Mevalonate, Cholesterol

BILE ACIDS

- Extra cholesterol is converted to Bile Acid

- Cholesterol $\xrightarrow[\text{[Peroxisome of liver]}]{7\alpha\text{ hydroxylase (Rate limiting enzyme)}}$ 7-hydroxy cholesterol

7-hydroxy cholesterol → Multiple steps → Cholic acid; Multiple steps → Chenodeoxycholic acid

Primary Bile acid ⟶ Cholic acid, Chenodeoxycholic acid

↓

Cause emulsification of lipids & breakage of large molecule into smaller

Terminal Ileum

↓

Secondary Bile acid [due to Bacterial action] → Deconjugation[Q]; 7α dehydroxylation[Q]

Cholic acid → Deoxycholic acid ↓ absorbed in Enterohepatic circulation

Chenodeoxycholic acid → Lithocholic acid ↓ Non absorbable, excreted from GIT

↓

Bile acids gets conjugated (in) cystic duct (glycine & Taurine 3 : 1) while going to Gall Bladder

Glycochenodeoxycholic acid

↑↑ Homocysteine → 1. associate c̄ Thrombosis, coronary artery disease, stroke
2. Osteoporosis & #
3. Neuropsychiatric disease
4. Developmental delay

AMINO ACIDS ⇒ 20 Standard Amino Acids

→ They are a group of organic compounds containing two functional groups → Amino & carboxyl.
↓ Basic ↓ Acidic

↑ Selenocysteine protein "P"

→ 21st AA → Selenocysteine → coded by UGA, stop codon
↳ Modified serine (-OH) Replaced by selenium
→ Structure similar to cysteine → Antioxidant
→ Prt at active site of - ① glutathione peroxidase
② Deiodinase;
③ Thioredoxin Reductase
④ Glycine Reductase

→

→ 22nd AA → Pyrrolysine → coded by UAG

→

$$COO^- - \overset{H}{\underset{R}{\overset{|}{\underset{|}{C^\alpha}}}} - NH_3^+$$

R → Varies in diff AA
[H or CH_3 or —]

"It ↑ the flexibility of the protein" ← glycine ⇒ Optically inactive (b/c of symmetric carbon)

→ Classification of AA :-
1. Based on structure;
2. Based on side chain character;
3. Based on Metabolic fate
4. Based on Nutritional Requirements

Gamma Amino Butyric Acid (GABA) is formed by "Decarboxylation of L-glutamate".

Enzyme involved is "glutamate decarboxylase (GAD)"

Pyridoxin dependent enzyme

Date
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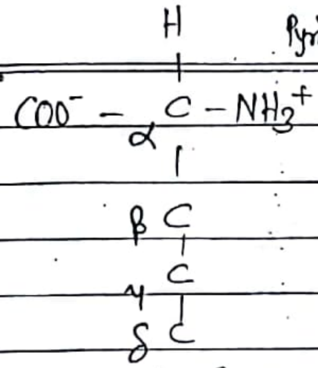

1. BASED ON STRUCTURE

(A) ALIPHATIC AMINO ACIDS :->

1. $\boxed{\text{Monoamino carboxylic acid}}$:->

- Simple -> Glycine, Alanine.
- Branched -> Valine, Leucine, Isoleucine (VIL)
- Hydroxyl -> Serine (on β-c), Threonine (on γ-c)
- Sulphur containing -> Cysteine (on β-c), Methionine (on γ-c)
- Amide group containing -> Glutamine, Aspargine

Monoamino dicarboxylic acid

2. $\boxed{\text{Monoamino dicarboxylic acid}}$:->

Aspartic acid (COO^- = α + β-c)

Most Acidic <- Glutamic acid (COO^- = α + γ-c)

Dibasic monocarboxylic acid

3. $\boxed{\text{Dibasic Monocarboxylic acid}}$:->

Arginine, Lysine, Histidine

Most Basic (Arginine) — Least Basic (Histidine)

Histidine has the Max^m buffering capacity at Physiological pH.

Aromatic Amino acids

(B) AROMATIC AMINO ACIDS :-> d/t it AA absorbs Light (UV).

Phenylalanine, Tyrosine, Tryptophane, Histidine

(Tryptophane) -> Max^m absorption of UV Light.

Heterocyclic amino acids

(C) HETEROCYCLIC AMINO ACIDS :->

Histidine, Tryptophan

Teacher's Signature ....

→ Modified (Non-standard) AA → Cystine; Hydroxy proline; Hydroxylysine, Desmosine & Selenocysteine.

(D) IMINO AMINO ACIDS :- Proline.

NH_2^{+}

(E) DERIVED AMINO ACIDS :- Hydroxyproline, citrulline, Homocysteine, hydroxylysine, ornithine

→ AMINO ACIDS | SPECIAL GROUP

AMINO ACIDS	SPECIAL GROUP
Arginine	Guanidinium - δ
Tryptophan	Indole - β
Histidine	Imidazole - β
Proline	Pyrrolidine - α
Tyrosine	Phenol - β
Phenylalanine	Benzene

"Sakaguchi test" is done for Arginine. identifies "Guanidium"

C-atom on which prf.

→ Function :-

1. Tyrosine — Catecholamine → Epinephrine, Norepinephrine, Dopamine
   — Thyroxine
   — Melanin
2. Tryptophan — Niacin
   — Melatonin, Serotonin
3. Arginine — NO
4. Glycine — Purine Base
5. Histidine — Best buffer at Physiological pH

Teacher's Signature ....

Absorption of light → By Aromatic AA (Maxm Tryptophan)
↓
→ Responsible for UV absorption in protein (250-290nm)

Date 27/2
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2. BASED ON SIDE CHAIN CHARACTER

↳ Location → Lipid Bilayer (Transmembrane)

(A) NON-POLAR :→ PPTTMILAV (HYDROPHOBIC), when present on surface; Polar in Nature

Phenylalanine; Proline; Tryptophan; Tyrosine; Methionine; Isoleucine; Leucine; Alanine; Valine

↳ Methyl group

(B) POLAR :→ Hydrophilic (out/inside membrane)

Uncharged → Glycine, Serine, Threonine, Cysteine

↳ May be Non-polar

Acidic → Aspartic acid, glutamic acid

Basic → Histidine, Lysine, Arginine

3. BASED ON METABOLIC FATE

(A) Purely ketogenic → *Leucine > Lysine

NEET 16' Ketogenic & glucogenic → Phenylalanine, Isoleucine, Tyrosine, Tryptophan (PITT)

(C) Glucogenic AA → Rest 14 AA are purely glucogenic

QQ

Amino Acids	Keto acids
Glutamate	→ αKG
Aspartate	→ OAA
Alanine	→ Pyruvate

Glutamate --> Alfa-KG

Aspartate -----> OAA

Alanine ---------> Pyruate

Teacher's Signature ....

Acetyl choline ⇒ Synthesized in the cytosol of Nerve terminal from Acetyl CoA & choline.

Acetyl co-A + choline $\xrightarrow[\text{acetyl transferase}]{\text{choline-O-}}$ Acetylcholine + coA

→ Not synthesized from Any AA.

Date ______ Page 87 (45)

4. Based on Nutritional Requirement

Essential	Non-Essential	Semiessential
Tryptophan	Rest 10	Argenine
Valine		Histidine
Metheonine		(Non-essential in
Isoleucine		grown-up child)
Leucine		(essential in pregnant
Argenine		old age, sick etc.)
Phenylalanine		
Threonine		
Histidine		
Lysine		

TV (IIMI) MILA PATH मे Le (mi)

UREA CYCLE | Ornithine cycle | Kreb's Henseleit cycle

- Occuring in hepatocyte
- Partly mitochondrial, Partly cytosolic
- Urea is formed in cytosol
- Mostly amino acids gives their Amino group to α KG to form Keto acid & glutamate

AA + α KG $\xrightarrow[\text{nase (vit. } B_6)]{\text{Transami-}}$ Keto acid + glutamate

(NH₂ transferred from AA to αKG)

* Two Mechanisms are available in human for the transport of NH_3 from Peripheral tissue to Liver ⇒ (1) Transport of Ammonia in form of Glutamine; by all tissues except Muscle;

Teacher's Signature..........

(2) Transport of Ammonia ... Alanine ... by Muscle.

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- Carbamoyl group of carbamoyl phosphate synthase is derived from "NH_4^+ & HCO_3^- ion."

* AA degradation ⇒ occurs in Reticuloendothelial cells

AA + αKG ⟶ Ketoacid + Glutamate

Glutamine Synthetase

$+NH_3$

ATP → ADP

Major donor of NH_3 in Mitochondria of Liver. ← Glutamine

Glutaminase

Liver ← NH_3 + Glutamate

Mitochondria

Brain is a Rich Source of Glutamine Synthetase & predominantly detoxifies NH_3 by synthesis of Glutamine

Glutamate — Glutamate dehydrogenase → αKG + NH_3

NADH

CO_2

Both Rxn K/a → Oxidative deamination → delivery of NH_3

allosterically activated by "N-Acetyl glutamate".

Mitochondria of hepatocytes

cytosol of hepatocytes

NH_3 + CO_2

2ATP → 2ADP

CPS-I → Rate limiting enzyme

⤷ carbamoyl phosphate synthase

Carbamoyl phosphate

ornithine transcarbamoylase | ornithine carbamoyl transferase

ornithine transporter (Antiport)

ornithine

Citrulline → Neither prt in tissue proteins nor in blood; but it is prt. in Milk.

ornithine transporter

Citrulline

ATP → AMP + PPi

Argininosuccinate Synthetase

+ AA

Aspartate

Argininosuccinate —Argininosuccinase→ Arginine

OR Argininosuccinate lyase

Fumarate ↳ goes to TCA cycle

Arginase

H_2O

$O=C\begin{matrix}-NH_2 \\ -NH_2\end{matrix}$ Urea

from NH_4^+

from Aspartate

* CPS-II → cytosolic enzyme involved in "pyrimidine synthesis".

Teacher's Signature

Aspartate $\xrightarrow[\text{ATP} \rightarrow \text{AMP + PPi}]{\text{Glutamine} \rightarrow \text{Glutamate}}$ Asparagine

Date ____
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→ Total 4 high energy phosphate is released here.

⇓

Fumalate

↓

Malate ⟷ OAA (NADH)

OAA + Glutamate (Source of NH_3 for Aspartate) $\xrightarrow{\text{SGOT (AST)}}$ Aspartate + α-KG

Occur in Mitochondrial Matrix of hepatocyte

Aspartate again enter urea cycle.

→ Few Reaction of TCA cycle is also required for Urea cycle.

Urea: $NH_2-CO-NH_2$

→ Urea → 2 ammonia required
1 → from glutamate → (free NH_3)
2 → combined c̄ aspartate → (Bound NH_3)

∴ Source of Nitrogen in urea → Aspartate, Ammonia

Source of 1st Carbon in Urea → CO_2.

UREA CYCLE DISORDER

Hyperammonemia → Lethargy; Nausea; Vomiting.

(1) Carbamoyl phosphate synthase I → Hyperammonemia type I

HHH Syndrome

(2) Ornithine transporter → HHH Syndrome NEET'16 Hyper
— Hyperammonemia; Hyperornithemia; Homocitrullinemia

Phenylalanine type I/II/III ⇒ d/t deficiency of Phenylalanine hydroxylase
Phenylalanine type IV ⇒ d/t deficiency of DHB ~~Reductase~~
Phenyl ketonuria type V ⇒ d/t deficiency of DHB. Synthase

③ Ornithine transcarbamoylase → ~~Hyperammonemia type II~~ — Less toxic then type I.
→ Orotic aciduria } X-linked Q

④ ArginoSuccinate Synthase → Citrullinemia

⑤ ArginoSuccinase / ArginoSuccinate Lyase → ArginoSuccinic aciduria
↓
associated with "friable & tufted hair"
k/as "Trichorrhexis Nodosa"

⑥ Arginase → Hyperargininemia
↓
Autosomal Recessive disease

b/c Arginine Reabsorbed ← { Lysine, ~~cysteine in urine~~
Arginine high in Blood

ⓐ Dc by exchange of Lysine & cystine.

→ Which AA is regenerated in Urea cycle → Ornithine!

CONVERSION OF PHENYLALANINE TO TYROSINE

Phenylalanine (essential AA)
- Vit. C
- O_2
- Cu^{+2}

Phenylalanine hydroxylase ⊕
THB ← DHB Reductase (NADP+ / NADPH) — 3 enzyme required
② Tetrahydrobiopterin
→ DHB → ③ Dihydrobiopterin
DHB synthase by acting on Biopterin.

↓
Tyrosine (Non-essential AA)

Phenyl Pyruvate → Phenyl lactate, Phenyl acetate
↓
Phenyl ketones
Release in urine due to defect of the Phenylalanine hydroxylase enzyme

Classical PKU — Phenylalanine hydroxylase deficiency
Phenyl Ketonuria —
{ Rx — Restriction (Not total) of Phenylalanine in diet (Life Long)
• Tyrosine should be supplemented

Mousy odour is due to Phenylacetate

1° Bile acid → cholic acid, chenodeoxycholic acid

2° Bile acid → deoxycholic; Lithocholic acid

Date
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(THB ⇒ donor of H in → ① PKU → Tyrosine
② Serotonin synthesis ③ Catecholamine synthesis

→ Atypical PKU → due to deficiency of "Dihydrobiopterin Reductase"

→ Screening of PKU → Guthrie test (bacterial inhibition assay);

$FeCl_3$ test (Urine) → green ⊕

TYROSINE

Melanin, Catecholamine, T_3, T_4, Tyramine

→ Cu^{2+} deficiency → will lead to melanin deficiency ⇒ Flag hair

Melanin Synthesis → Tyrosine
↓ Tyrosinase* ← Rate Limiting enzyme for Melanin synthesis
↳ Cu^{2+} containing enzyme
DOPA (Dihydroxyphenylalanine)
↓ Tyrosinase
Dopaquinone
↓
Menaquinone
↓
Indole quinone
↓
Melanin (Pigment)

NEET '16 Q.

Albinism
↓
Tyrosinase deficiency (Mutation of the gene of Tyrosinase)

QQ Leucoderma (vitiligo)
⇓
Antibodies against Melanoblast
Patchy loss of Pigment

Teacher's Signature

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→ creatine & creatinine → Syn from glycine, Arginine, Methionine

↑ VMA in Urine ⇒ Pheochromocytoma

Catecholamine Synthesis →

Tyrosine
↓ Tyrosine hydroxylase* → RLE (Rate Limiting enzyme)
DOPA
↓ Dopacarboxylase
dopamine
↓ Dopamine β-hydroxylase
Norepinephrine
↓ N-methyl transferase (SAM → SAH)
epinephrine

Methyl Donor ↑ SAM

SAM — S-adenosyl methionine

SAH — S-Adenosyl homocysteine

Catecholamine → Metanephrine → VMA (vanillyl mandelic acid) → excreted in urine 2-6mg/24hr

Tyrosine catabolism

Tyrosine
↓ Tyrosine transaminase (αKG → Glutamate) ⊗ Tyrosinemia II (Richer Hanbort Syndrome) — NEET'16

Richer Hanbort syndrome

Parahydroxy phenyl pyruvate
↓ Hydroxylase Decarboxylase (O_2 → CO_2) ⊗ Tyrosinemia III / Neonatal tyrosinemia
2,5- dihydroxy phenyl acetic acid (Homogentisic acid)
↓

OCHRONOSIS → the cond^n in which alkapton deposition occurs in connective tissue, bones & various organs (Nose, ear etc.)

Date ___
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↓ Homogentisic acid oxidase (X) Alkaptonuria
↳ Black urine on standing
· associated c̄ "OCHRONOSIS"

Maleyl acetoacetic acid
↕ Isomerase
Fumaryl acetoacetic acid

Hydrolase — Fumarylacetoacetate hydrolase (X) Tyrosinemia I → cabbage odour urine (NEET 18)

Fumaric acid → glucose
Acetoacetic acid → Ketone body

By producing "Alanine"
By producing "Acetyl Co-A"

60 mg tryptophan → 1 mg Niacin

$\boxed{\text{TRYPTOPHAN}}$ both glucogenic & ketogenic
→ first identified essential AA
→ Precursor for the synthesis of important compounds like NAD+, NADP+, Serotonin, Melatonin.

Tryptophan
THB → DHB
↓ Tryptophan hydroxylase
5-Hydroxy tryptophan
↓ Decarboxylase
5-hydroxy tryptamine [Serotonin] → 5-hydroxy indole acetic acid (HIAA)
↓ Plasma / Urine
↓ Inc in carcinoid syndrome
↳ Argentaffin cell proliferate

5-hydroxy tryptamine [Serotonin]
↓ Acetyl transferase
Acetyl serotonin
↓ Methyl transferase
Melatonin → Pineal gland hormone

Teacher's Signature ....

→ NAD$^+$ Pathway | Kynurenine Pathway or Major metabolic pathway of tryptophan ⟶

It is a catabolic pathway

Tryptophan
↓
N-Formyl kynurenine
↓
Kynurenine
↓ 3-OH kynurenine
B_6 def ⫲ Kynureinase [PLP | B_6]
↓

QQ Xanthurenic acid — 3-hydroxy — Anthranillic acid

50% NAD form from Niacin

Also form from Niacin (Vit. B_3) (50%) → NAD$^+$ (3%) — Ketone body (97%)

↑ Rest 50% from Tryptophan (B_6)

In B_6 deficiency → ↑ Kynurenine
→ ↑ 3-OH Kynurenine
⇓
B_6 Pellegra ⇒ deficiency of B_6; → xanthurenic acid excreted in urine
that have Pellegra like Manifestation (↑B_6 def - index)

Tryptophan Load test → done to find out latent B_6 deficiency

Hartnup disease → Tryptophan metabolism defect

Hartnup disease — Tryptophane metabolic defect

* Pellegra ⇒ Vit. B_3 deficiency

Date
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- Taurine is biosynthesized by "cysteine"! Q
- AA Secreted in cystinuria are → C → cystine, O → ornithine, L → Lysine, A → Arginine

METHIONINE

Sulphur containing essential AA.

Features of Homocystinuria:-
- Downward subluxation of Lens
- Aortic Aneurysm
- Skeletal Deformities
- Arachanodactyly.

B_{12} supplement
↑ T/t
Homocysteinuria type II

Methionine
↓ ATP → $PP_i + P_i$ (Adenosine Left)
S-adenosylmethionine (SAM)
↓ Acceptor → Methylated product
S-adenosylhomocysteine (SAH)
↓
Homocysteine
↓ Serine → cystathionine β-synthase [PLP | B_6]
Cystathionine
↓ cystathionase [PLP/B_6] → cystathioniuria
Cysteine + Homoserine

Homocysteine | Methionine methyltransferase | synthase (B_{12} dependent)
N^5-Methyl THF → THF; B_{12} → CH_3-B_{12} (Homocysteine → Methionine)

Typical/classical homocystinuria
⇑
Homocysteinuria type I
↓
R_x → B_6 supplement
cysteine becomes essential AA; b/c Not formed

↑ Homocysteine a/w "Atherosclerosis & cognitive distortion"

- "Methionine & cysteine" both required for proper wound Healing".
- NEET'16 Molecular Mimicry is established in presence of "cysteine; Arginine or Lysine".

~~Cysteine → Serine → Methionine~~

Cysteine -> serine -> Methionine

Cystine → dimer of cysteine (disulfide bond b/w two cysteine Molecules) Q

Folate trap → due to B_{12} deficiency.
N_5 Methyl THF ✗→ THF
So, folate trapped in N_5 methyl THF (i.e. methylated) form.

- Beta Alanine → End product of cysteine.

Teacher's Signature ....

Beta Alanine -> End product of cysteine

Conversion of Glycine to Serine →

(VVS) NEET'16

Glycine + THF $\xrightarrow[\text{transferase, PLP}]{\text{Serine hydroxymethyl}}$ Serine + N^5, N^{10} – Methylene THF + H_2O

dUMP $\xrightarrow{\text{Thymidylate synthase}}$ TMP → DNA

$N^5 N^{10}$ Methylene THF → DHFA

↓

This enzyme requires $N^5 N^{10}$ methyl THF

↓

Not formed in folate trap

↓

∴ DNA synthesis hampered due to inhibition of above reaction

↓

Cell enlarges but No nuclear division

↓

Megaloblastic Anaemia

NEET'16

BRANCHED CHAIN AA

Leucine — Valine — Isoleucine

↓ Transaminase (multifunctional)

↑↑ α-Keto acid — ↑↑ α-Keto acid — ↑↑ α-Keto acid

↓ Branched chain α keto acid decarboxylase or dehydrogenase complex (oxidative decarboxylation) (3 enzyme; 5 co-enzymes)

Isovaleryl CoA — Isobutyryl CoA — α-methyl butyryl CoA

↓ Isovaleryl CoA Dehydrogenase — ↓ Isobutyryl CoA Dehydrogenase — ↓ α-methyl Butyryl CoA dehydrogenase

X — Y — Z

HMG CoA → Acetyl CoA — Propionyl CoA — Acetyl CoA + Propionyl

Teacher's Signature ....

Maple Syrup urine disease (MSUD) / Branched chain Ketonuria :→

- deficiency of Branched chain α Keto acid decarboxylase complex
↓
Result in Branched chain Ketonuria
↓
excretion of α Keto acids in urine

- Urine odour → Burnt Sugar / Maple syrup.

Isovaleric acidemia :- deficiency of Isovaleryl CoA dehydrogenase
↓
Sweaty feet odour · Leucine catabolism defect

- Urine odour → Cheesy odour (Isovaleric aciduria)
↳ Isovaleric Aciduria

→ Order of Rx^n :→ Transamination
↓
Oxidative decarboxylation
↓
Dehydrogenation

HISTIDINE

Histidine catabolism :→ Histidine
↓ Histidase ✗
Benign Histidinemia
Urocanic acid
↓

Teacher's Signature ....

CET MQ '15

Histidine $\xrightarrow[\text{PLP}]{\text{Histidine Decarboxylase;}}$ Histamine + CO_2

Urocanic acidyria ← ✗ Urocanase

Imidazolone Propionic acid

↓ 4-imidazolone 5-Propionase

H_2O →

FIGLU (Formimino-glutamic acid)

↓ THF

Folic Acid deficiency ✗

→ N^5-Formimino-THF

Glutamic acid

Q. FIGLU in Urine → def. of folic acid

• Histidine load test → to find out latent folic acid deficiency

→ Urinary odours in Biochemical Disorder :-

Inborn error of metabolism	Urine odour
1. Multiple carboxylase deficiency	Tomcat Urine
2. Phenylketonuria	Musty & Mouse like Q
3. Tyrosinemia	cabbage like; Musty
4. Hypermethioninemia	Boiled cabbage Q, Rancid butter Q
5. Trimethylaminuria	Rotting fish
6. Oasthouse Syndrome (Methionine Malabsorption)	Sweet (Dried malt)
7. Maple Syrup Urine Disease	Maple syrup, Burnt Sugar
8. Glutaricacidemia (type II)	Sweaty feet, acrid
9. Isovaleric acidemia	Sweaty feet, acrid
10. Hawkinsinuria	Swimming Pool

Teacher's Signature ....

Edman's Reagent → Phenyl-isothiocyanate
Sanger's Reagent → 1-Fluro-2,4-dinitrobenzene

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STRUCTURE OF PROTEIN

1° → Covalent Peptide bond
2° → Hydrogen bond

α-Helix → M/c 2° structure
↳ Rt handed helix → more stable (M/c)

Proline > Glycine → Never form α-Helix
↳ disrupt α-helix.

β-pleated → glycine → M/c Amino acid to kink

A tight curl or twist.

3° → Polypeptide chain → weak Non-covalent interaction

4° → Polymer of polypeptide chain
eg → Immunoglobulin
Protein structure

Simple Protein
- Fibrous Protein → Collagen, Keratin, Myosin, Elastine
  contains Histidine, Lysine, Arginine in 1:4:12 Ratio.
- Globular Protein → Albumin, Globulin, Glutelin, Prolamine, Histone, Lectin, Protamine, Globin

Conjugated Protein → Nucleoprotein, Lipoprotein, Phosphoprotein, Chromoprotein, Metalloprotein, Glycoprotein

Derived Protein → egg white (coagulated albumin), Proteans, Metaproteins
Proteoses, Peptones, Polypeptides, Peptides

Teacher's Signature ....

Date 28/12
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Ultra centrifugation } → Base of size
Ultra-filtration

→ Loss of $2^\circ, 3^\circ, 4^\circ$ structure of Protein.

Agent ← Denaturation → Loss of Biological function of Protein.
Urea or ⇓
M guanidine chloride: Irreversible ; Peptide bond (covalent bond) remains Intact.

→ Chromatography → ① Gel filtration → Based on size
↳ Mass movement by diffusion.

② Ion exchange → Based on charge

→ Electrophoresis → Based on charge & Mw
↳ M/c method of Protein separation.

PAGE (Polyacrylamide gel electrophoresis)
↳ Based on Mol. size & Mol. charge

sodium dodecyl sulfate
SDS - PAGE
↳ for Mw determination

Protein gradient created by → Ampholyte

Cystine → formed by disulfide bond b/w two cysteine

→ Cysteine & Cystine formed from → Methionine
→ Serine.

→ Glycine → Glycine $\xrightarrow{THFA, PLP}$ Serine
↳ Synthesis of Heme, Purine Ring, Bile acid conjugation.

→ Serine → Cysteine & Selenocysteine formation

Carnitine → from Lysine & Methionine

Teacher's Signature ....

- Ninhydrin Test → all α-Amino acids;
- Xanthoproteic Rxn → Aromatic amino acids;
- Millon's Test → Tyrosine (Phenol gp. of Tyrosine);
- Aldehyde test → Tryptophan;
- Biuret's test → Peptide bond;

conc^n. Nitric oxide used.

- Diazo Rxn → Histidine or Tyrosine

Keratin → Histidine + Lysine + Arginine (HLA)
1 : 4 : 12

- Alanine → from Pyruvate
- Aspartate → from OAA.
- Glutamate → from αKG
- NEET'16 Proline → from αKG

→ Smallest functional unit of genome → gene

Smallest unit of genetic expression → cistrone

Nucleotide & its metabolism

Nucleotide → N₂ Base, Pentose Sugar, Phosphate (one/two/three)

Pentose Sugar → Ribose, Deoxyribose

Nucleoside → Nucleotide − PO_4^{-2}

N₂ Base N2 Base

N₂ Base → Purine Base, Pyrimidine Base

Purine Base
- Adenine
- Guanine & more old.

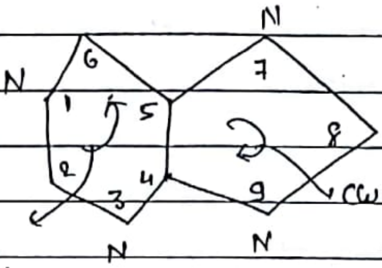
⇓ double cyclic structure

Pyrimidine Base
- Cytosine
- Thymine
- Uracil

⇓ Single cyclic structure

Teacher's Signature ....

Purine Base

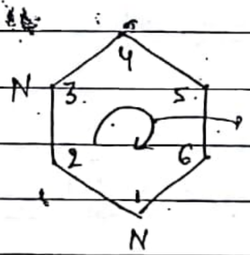


N → at 1,3,7,9 position.

cw in the 2nd Ring

Acw In the 1st Ring.

Pyramidine Base



N → at 1,3 position

clockwise

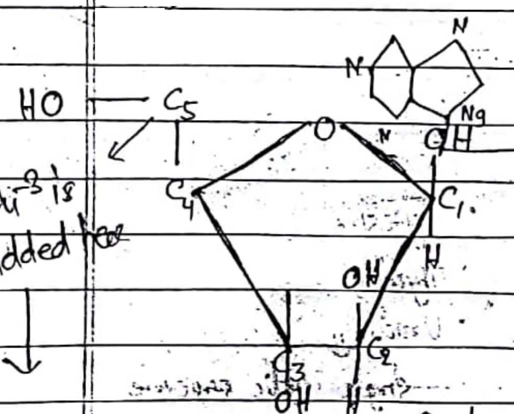
Adenine → 6 amino purine

Guanine → 2 amino, 6 oxy purine

Cytosine → 2 oxy, 4 amino Pyrimidine

Thymine → 2,4-dioxy 5 methyl pyrimidine

Uracil → 2,4- dioxy pyrimidine



→ β-N glycosidic bond NEET'16

↓

b/w sugar & base

PO_4^{-3} is added here

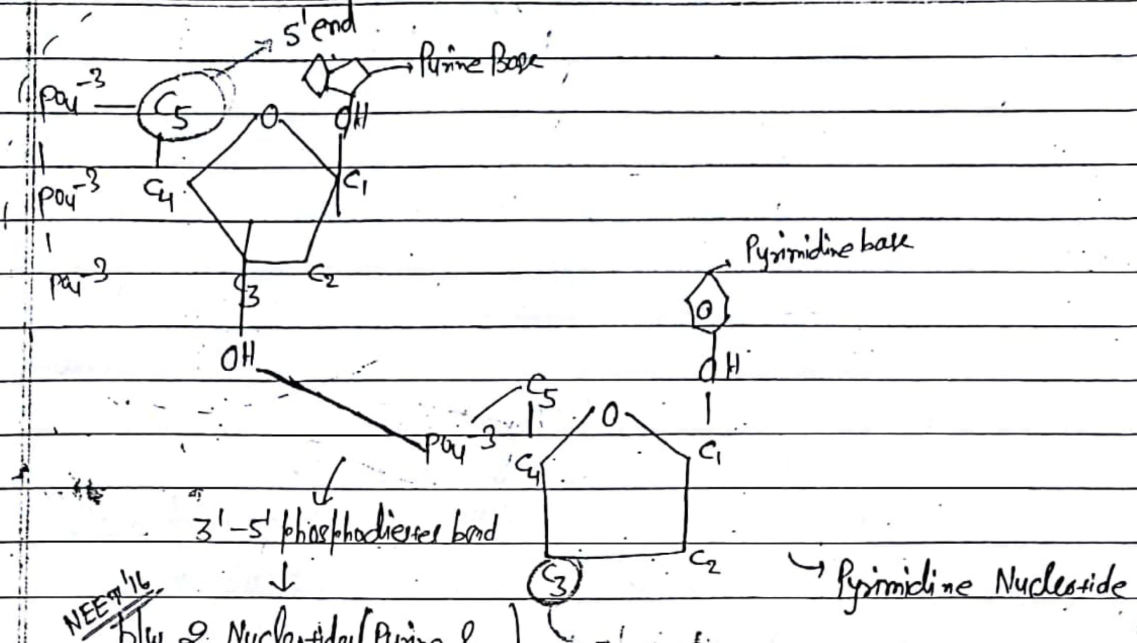
↓

Esterification

Ribose $\xrightarrow[\text{at } C_2]{O_2 \text{ absent}}$ deoxyribose

M|C → B-DNA → Rt. handed

→ Purine Nucleotide



↓

NEET'16 b/w 2 Nucleotides (Purine & Pyrimidine)

free end is called → Prime end

∴ Direction of phosphodiester bond is 3'-5'
3' → Hydroxyl
5' → PO_4^{-3} end

NUCLEOTIDE METABOLISM

→ "Purine Nucleotide"

- Synthesis
  - → Denovo
    - Glycine → C_4, C_5, N_7
    - Aspartate → N_1
    - Glutamine → N_3 & N_9 (1st give N_9 & then give N_3)
    - THF derivatives → C_2 & C_8 → N^{10}-Formyl THF, N^5-N^{10} Methenyl THF
    - CO_2 → C_6
  - → Salvage pathway ↓ Reutilization of bases to form purine
- Break down

* "PRPP glutamyl amidotransferase" is one of the rate limiting enzyme in denovo synthesis of Purine.

* Denovo synthesis is most active in liver. Major Pathway

"Liver" → Major site of Purine Nucleotide biosynthesis (De Novo).

Brain, erythrocytes & PMNL can't synthesize Purine Nucleotides by de novo pathway. ∴ salvage pathway synthesize purine here.

PRPP → Phosphoribosyl Pyrophosphate

Salvage → Recovering, saved from loss.

Salvage Pathway (Rare)

The Purine can be directly converted to the corresponding nucleotides.

→ Source of Purine → Diet (1°)
DNA, RNA breakdown (2°)

Phosphoribosyl-5-Pa

↓ PRPP Synthetase

Diet

Adenine + PRPP $\xrightarrow{\text{Adenine Phosphoribosyl transferase}}$ AMP

Guanine + PRPP $\xrightarrow{\text{HGPRT}}$ GMP

Purine catabolism → Hypoxanthine + PRPP $\xrightarrow{\text{HGPRT}}$ IMP

HGPRT → Hypoxanthine guanine phosphoribosyl transferase
↳ Bi-functional enzyme.

Salvage is significant in → RBC, Brain.

Deficiency of HGPRT → Lesch-Nyhan Syndrome

Catabolism of Purine

Lesch-Nyhan Syndrome → complete deficiency of HGPRT.
↳ Sex linked metabolic disorder
↳ Structural gene for HGPRT is located on X-chromosome
↳ affects only the males
↳ C/F → Excessive uric acid production
Neurological manifestation (Mental Retardation, Aggressive behaviour, Self Mutilation)

Teacher's Signature

- AlloPurinol ⇔ ~~Hypoxanthine Analogue~~.
- ~~Purine is structural component of~~
  ⇓
  ~~Coenzyme-A, NAD^+, $NADP^+$ & FAD^+.~~

Severe combined immunodeficiency

PURINE CATABOLISM

Adenosine

$\downarrow$ Adenosine deaminase [ADA] (X) SCID

⇓

T-cell & B-cell function decline

NH_3

Inosine

Pi

$\downarrow$ Purine Nucleoside Phosphorylase (X) T-cell decline & B-cell Normal.

Ribose-1-Phosphate

Hypoxanthine

HGPRT

GMP

$\downarrow$

Salvage

Xanthine oxidase (X) xanthinuria

Guanosine

$\downarrow$ Purine Nucleoside Phosphorylase

Guanine

H_2O

Guanase

NH_3

Xanthine

$\downarrow$ Xanthine oxidase (X) xanthinuria → Result in xanthinlithia

Uric acid → end product in human

$\downarrow$ Uricase (only in Birds & Reptiles OR Non-Primate Mammals)

Allantoin

Kelley-Seegmiller Syndrome → Partial deficiency of HGPRT.

No Self mutilation

only feature → Hyperuricemia

- Most of the dietary purines are converted to uric acid in the "intestinal mucosal cell only." Intestinal bacterial flora is involved in degradation of the rest of the dietary purines that remain absorbed.

* De-Novo Pathway of Purine synthesis: Ribose-5-P

Occur in Liver

ATP → AMP; $\downarrow$ PRPP synthase

PRPP

Glutamine → Glutamate; $\downarrow$ PRPP Glutamyl Amido transferase → Rate Limiting enzyme (Committed step)

5-Phospho-D-Ribosylamine ↑ (−)

AMP: 1st nucleotide to be formed

AMP

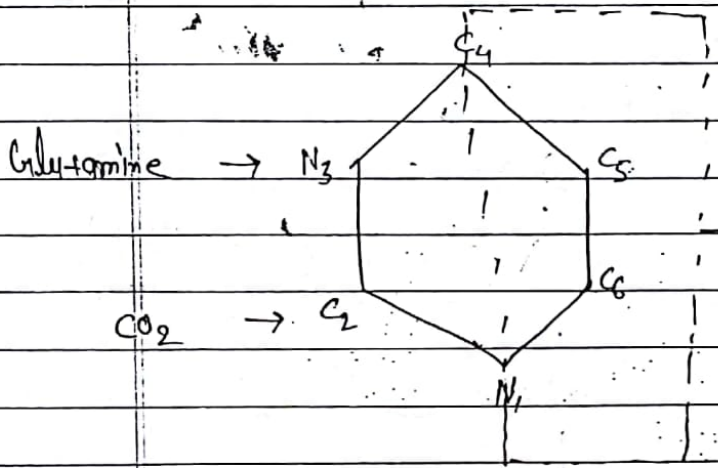
GMP

Pyrimidine Nucleotide

→ Main Synthesis is by denovo (also in Brain, RBC)

→ Salvage is very Rare (also absent in diet)

→ Precursor molecule → CO_2 & glutamine *



Denovo Synthesis → Resembles Urea cycle

CO_2 + Glutamine

↓ CPS-II ← ⊖ UTP, ⊕ PRPP

Carbamoyl phosphate

↓ Aspartate transcarbamoylase [ATC] ← ⊖ CTP / ⊕ ATP

Carbamoyl aspartic acid

↓ Dihydro-orotase

Dihydroorotic acid [DHOA]

↓ Dehydrogenase

Orotic acid

↓

Regulation of Pyrimidine Nucleotide synthesis :-

(A) Carbamoyl phosphate synthase-II

(B) Aspartate transcarbamoylase

in Prokaryote Aspartate

Eukaryote (in)

trick in examination

Isoenzyme → Same in Biochemical Catalysis of Rxn
↓ ↳ substrate Same
Rest all different

Date
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PRPP → orotate phosphoribosyl transferase (Q)

OMP (orotidine monophosphate)

Orotidylate decarboxylase (Q) → CO_2

Part of UMP synthase → Bifunctional enzyme

$\boxed{UMP}$ → Common Precursor for Pyrimidine (Q)

UMP → UTP; UMP → dUMP (Reductase)

UTP → CTP (Amino-transferase)

dUMP → TMP (Thymidylate Synthetase; N^5,N^{10}-Methylene H_4 folate → H_2 folate) (QQ)

Orotic Aciduria

Type I

- deficiency of orotate phosphoribosyl transferase
+
deficiency of orotidylate decarboxylase

Type II

- deficiency of only orotidylate decarboxylase

CET-JULY'16: Deficiency of folate or vit B_{12} can cause hematological change similar to orotic aciduria ✓

Catabolism of Pyrimidine

dUMP, CMP → β-alanine + CO_2 + NH_3

TMP → β-amino isobutyrate + CO_2 + NH_3 (NEET'16)

water soluble

So, no associated disease as even in their excess, they are excreted

Teacher's Signature ....

Mitochondrial DNA

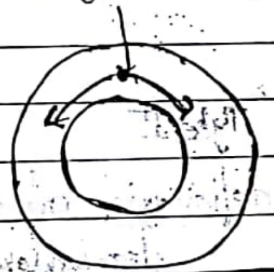
- 1% of total cellular DNA lies in Mitochondria
- Consist of 2-10 copies of Small circular DNAs
- mtDNA codes for 13 protein that play a key role in Respiratory chain (Rest all protein from Nuclear DNA).

AIIMS → 17 May → Replication without Proof reading
↓
∴ More chances of Mutation

Maternal inheritence

Replication of DNA

ori



Prokaryotic DNA | Eukaryotic DNA

Ori → Single
↓
origin of Replication

Multiple ori

- Replication from ori is Bidirectional in Both.

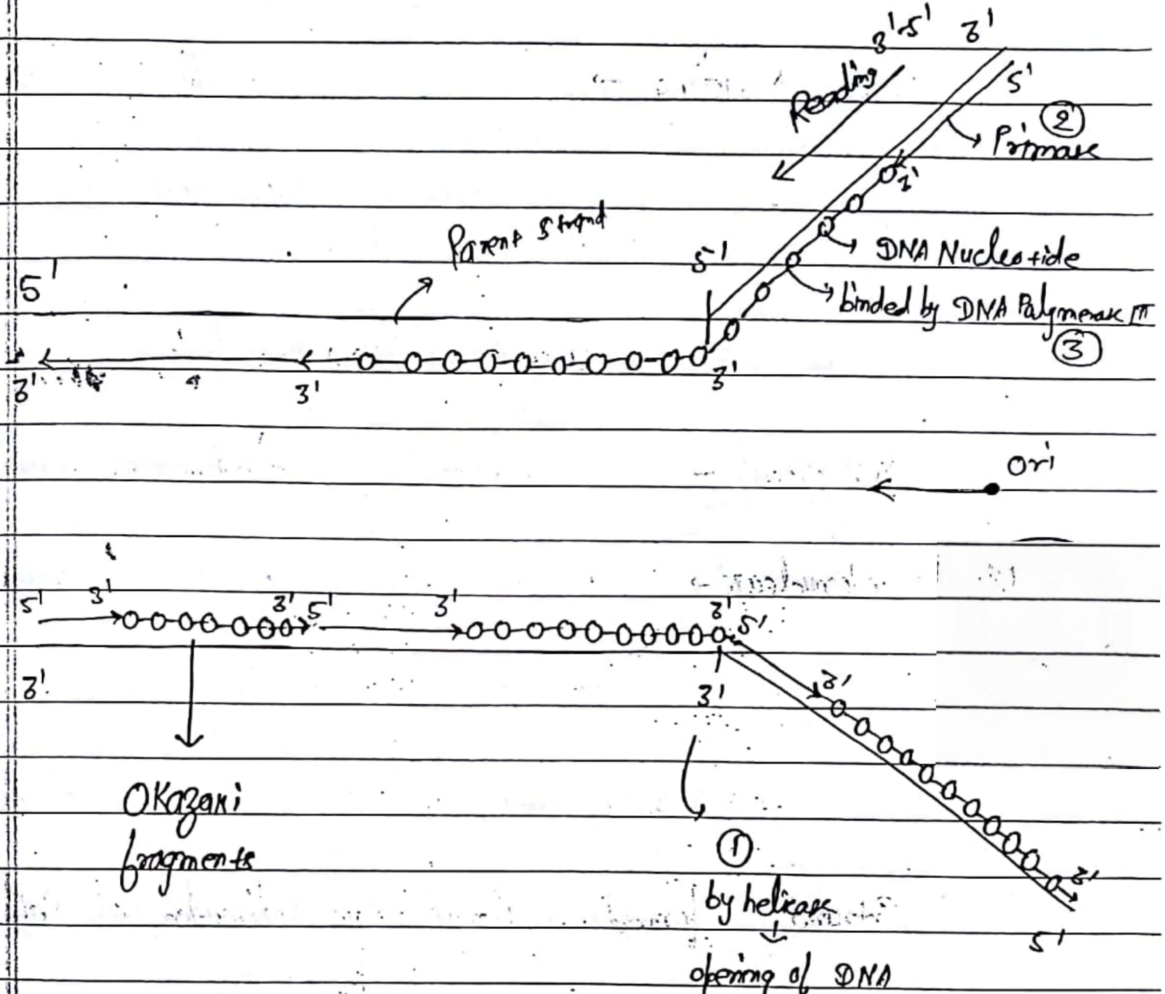
Replisome

Complex of protein & enzymes for the replication

Teacher's Signature ....

- DNA Replication occurs in 3 phases → Initiation, elongation & Termination.
- E. coli (Bacteria) has single, closed, circular, ds DNA; which is negatively supercoiled prt. in Nucleoid.

→ Segmental breaking (opening) & Segmental Synthesis of DNA occurs.



- Primase will form RNA Primer
  ↳ DNA dependent RNA Polymerase
- Reading of Nucleotide of Parent Strand will be in a direction specific manner → 3'-5'
- Synthesis of RNA/DNA → along 5'-3' direction.
- DNA Polymerase III → shows 2 catalytic activity
  (1) 5'-3' Polymerase → (2) Synthesis of daughter DNA
  ↓ addition of Nucleotide

Teacher's Signature

Proof Reading → Intra-Replication
DNA Repair → Post-Replication

Date
Page 110

② 3'-5' Exonuclease
↳ Proof reading

- Proof Reading → During Synthesis of DNA if any wrong nucleotide is added, then it is removed immediately by DNA Polymerase III in 3'-5' dirn.

– absent in RNA formation.

- Exonuclease → Cutting of External phosphodiester bond
- Endonuclease → Cutting of internal phosphodiester bond

5' O | O | O / O | O 3'
Endonuclease
5'-3' Exonuclease — 3'-5' Exonuclease

- Strand formed along the direction of Replisome
↳ Leading Strand

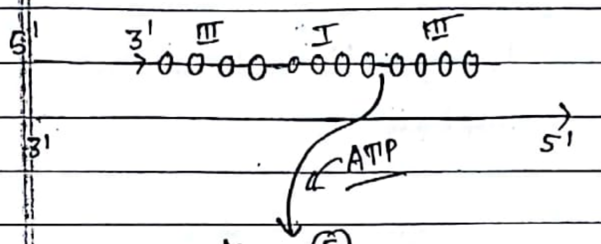
- Strand formed opposite the direction of Replisome
↳ Lagging Strand

- DNA Replication is → Semiconservative & Continuous

Kornberg enzyme/
- Removal of RNA Primer → by DNA Polymerase I

Gap filling ← ① 5'-3' Polymerase activity } (+) in DNA Polymerase I/II/III
Proof Reading ← ② 3'-5' Exonuclease activity }
③ 5'-3' Exonuclease activity
↳ Removal of RNA Primer

Klenow fragment → When 5'→3' exonuclease activity is Removed; Remaining Large fragment of DNA Polymerase I is k/as "KLENOW FRAGMENT".



Ligase (5)
↓
joins the fragment formed by DP III & DP I [By phosphodiester bond]

• Gap filling after Primer Removal
↓
by DNA Polymerase I (4)
↓
& Proof reading is also done by DPI here.

Okazaki fragments = DNA + RNA Primer
↓ ↓
formed by — DNA Polymerase III + Primase

DNA Polymerase II = Repair activity
↳ Not Involved in DNA Replication

Replisome :→

1. Helicase → Unwinding of DNA (Parent strand)
2. SSBP → Prevent premature annealing of ds DNA
(Single Strand Binding Protein)
3. Topoisomerase → Relieves torsional strain (supercoiling)
4. DNA Primase → RNA Primer Synthesis
↳ DNA dependent RNA Polymerase.
5. DNA Polymerase → DNA Polymerisation
↳ III / I
6. DNA ligase → Nick sealing. ATP is required.

Teacher's Signature ....

⊖ve Supercoil → direction of coil is opposite to Base coiling

⊕ve supercoil → No. of coils ↑ in same dirn of Basal coil

Date
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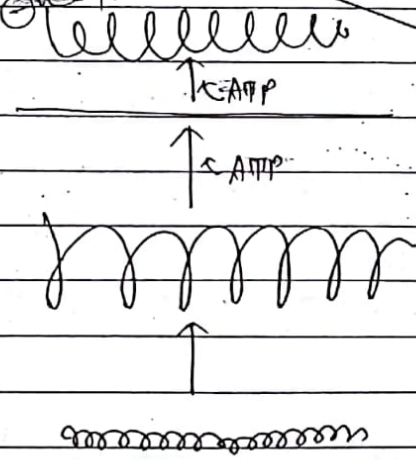
- DNA ligase → forms phosphodiester bond to join deoxy-nucleotides.

- Topoisomerase → Prevents supercoiling
  ↓
  So, that DNA replication can occur
  (activity of Helicase is smoothly occuring)

TOPOISOMERASE

I	II
Relieve ⊖ve Supercoils	Relieve +ve supercoils

Bacteria
↓
DNA gyrase
↓
Topoisomerase II



- Introduce ⊖ve Supercoil (someimes)
- No ATP required | ATP required for introducing of ⊖ve supercoil (i.e. sometimes ATP req)
- Nicks Single strand of DNA strand | ← Nicks both strand of DNA.

- Topoisomerase III → introduce single strand break during Recombination [redacted]

DNA Polymerase

E. coli [Prokaryote]	Mammalian [Eukaryote]	Function
I	α	Gap filling, synthesis of lagging strand
	ε	DNA Proof reading & Repair
II	β	DNA Repair
	γ	Mt DNA Synthesis $^{AI \to B}$
III	δ	• Processive Leading strand Synthesis • Okazaki fragment Synthesis

Eukaryotes →

α & ε → Lagging strand Synthesis

δ → Leading strand Synthesis

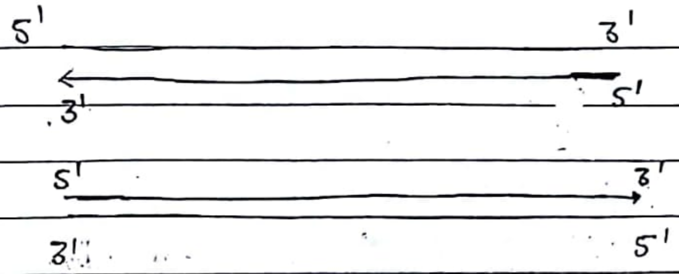
Proof reading & Repair activity present with all DNA Polymerase.

Telomere & Telomerase

- Arrangement that prevents the loss of daughter DNA.
- only present in eukaryotic DNA
- In Eukaryotes shortening of daughter strands occur ē each

Teacher's Signature ....

replication.



due to lack of receeding (Peeche) Nucleotide at extreme ends gap filling is absent.

↓

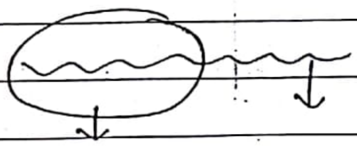
hence Shortening occurs.

→ 6 Nucleotide DNA segment → Hexanucleotide

↓ 5' TTAGGG 3'

↓ Telomere

→ Telomerase →



Protein factors + RNA (itself)

↓

i.e No enzymatic action

(RNA dependent DNA Polymerase)

Reverse transcriptase activity of Telomerase

↓

Present in protein factors

• Telomerase is not a ribozyme

• $\boxed{\text{Telomere} \rightarrow 5'\ TTAGGG\ 3'}$

↳ Rich in guanine [G-rich]

RNA of telomerase provides template for telomere formation.

Date 01/03 Page 115 (59)

- Telomerase is abs. from Most Somatic cells. It is prs. In germ(germinal cells, Cancer cells & Stem cells (hematopoietic stem cells)

* As the advancing age; Loss of enzyme activity $\bar{c}$ advancing age

- Telomerase is added on 3'-end of New Daughter DNA (20–200 telomeres)

↓

Seen as overhanging segment of Newly synthesized DNA.

- Telomere added on 3' end of Parent DNA strand

5' ——— 3'

3' ——— 5'

Telomerase

5' ——— 3'

← CAAUCCCAAUC

TTAGGGTTAGGG → 3' ——— 5'

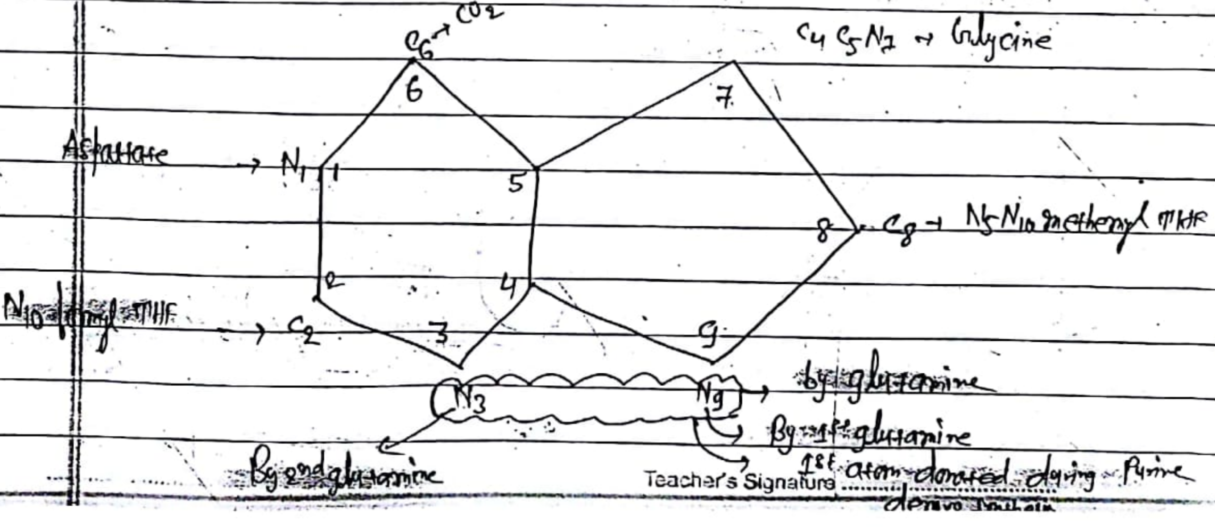
Telomer (work as Primer)

- Shortening is prevented b/c the end which is not replicated from parent was also telomere

* In certain Malignancies ⇒ Telomerase gene Mutated ⇒ Persistent Telomerase activity ⇒ Multiple Replication

De Novo Synthesis

- It refers to Synthesis of complex molecules from simple molecules. eg → Nucleotides are not needed in the diet as they can be constructed from small precursor molecules



Teacher's Signature

α-D Ribose-5-PO₄

↓ PRPP Synthetase

donates N₉

PRPP

Glutamine → glutamate — PRPP glutamine amido transferase → RLE

↓

5 Phospho D Ribosylamine

↓

IMP → AMP, GMP

various atom

AMP ⊖, GMP ⊖ → feedback inhibition by AMP & GMP.

IMP

↓

Hypoxanthine

↓

1st Base formed

↓

then it forms adenine, guanine

Base	Nucleoside	Nucleotide
Adenine	Adenosine	AMP
Hypoxanthine	Guanosine	GMP.

Teacher's Signature ....

DNA Repair

→ Post Replication process ;

→ Mechanism :- ① Base excision Repair ;

② Nucleotide Repair ;

③ Mismatch Repair ;

④ Double-strand break Repair

MISMATCH REPAIR

only Repair in which demarcation of Parent & daughter required ← MISMATCH REPAIR → Immediate Postreplication Repair

Methylation done by Dam Methylase enzyme

3' —— CH_3 (A) —— CH_3 (A) —— 5'

5' —————— 3'

↓ Single strand cut by GATC endonuclease
↳ cut at GATC sequence

3' —— CH_3 —— CH_3 —— 5'

5' —— ( ) —— 3'

↓ (GATC EndoNuclease), from Near by the wrong Mismatch
Exonuclease - gap created

3' —— CH_3 —— CH_3 —— 5'

5' —— 3'

↓ DNA Polymerase III (defect Repaired)

3' —— CH_3 —— CH_3 —— 5'

5' —— 3'

↓

Teacher's Signature ....

↓ Repaired by ligase

3' ——— CH_3 ——— CH_3 ——— 5'

5' —————————————— 3'

↳ New strand without any defect

→ The template strand of the DNA exists in a methylated form, while the newly synthesized is not methylated.

Eg →

3' — A(CH_3) C G T C G A(CH_3) — 5'

5' — T G C ~~G~~ G C T — 3'

Methylation at adenine base → Parent strand

Methylation occurs by → Dam Methylase & only in Parent strand during Replication

↓

DNA adenine Methylation

HNPCC (Hereditary Nonpolyposis colon cancer)

↳ due to defective mismatch Repair System.

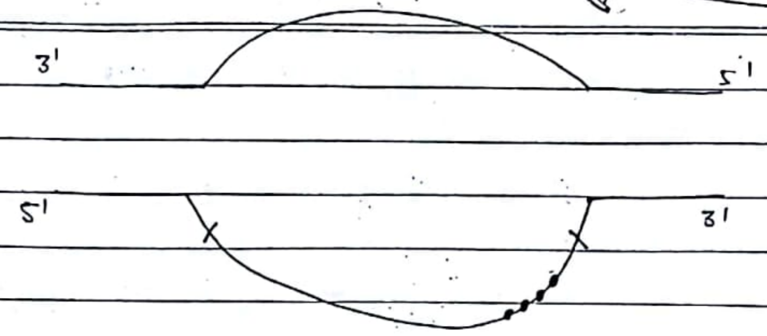
UV Light } Nucleotide Excision - Repair

3' —|———————|— 5'

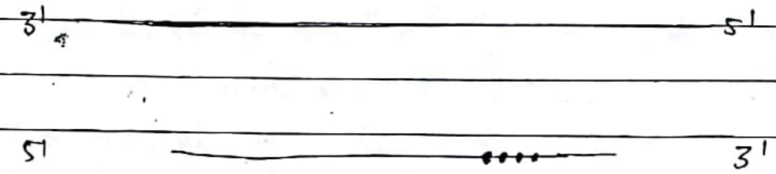
5' —|———————●●●●|— 3'

~~Cut by~~ ~~exonuclease~~ (excision nuclease)

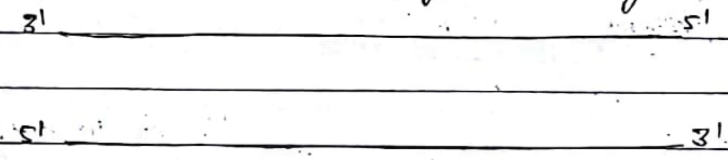
↓ Recognition & Unwinding



↓ Oligonucleotide excision by cutting @ 28 nts



↓ Degradation of defective DNA
Resynthesis & Religation



<u>Xeroderma Pigmentosum (XP)</u> → <u>defective Nucleotide excision Repair.</u>

U.V. light
covalent bond

5' A G T C A T C C A C G T 3'

3' T C A G T A G G T G C A 5'

Covalent linking occurs b/w Thymine dimers (Adduct) > Cytosine dimers
⇓
Pyrimidine dimer.

→ Autosomal Recessive
→ Accumulation of Thymine dimers;
→ ↑ Skin Malignancy (U.V. light); Blistering
→ Other malignancies also ↑ (Internal)

Intern's Signature ....

"Cockayne Syndrome" ⇒ also seen c̄ Nucleotide excision Repair

During Nucleotide excision Repair

↓

Cut made at 5' & 3' end

→ 20-25 bases are excised

→ done by UvrA, B, C (reactive) exinuclease enzyme

↓

Max^m case of Xeroderma Pigmentosum is due to its effect

Normally Not pr. in cell; but after UV Ray incident; it created & activated

Cut at two sites

Induced by U.V. light

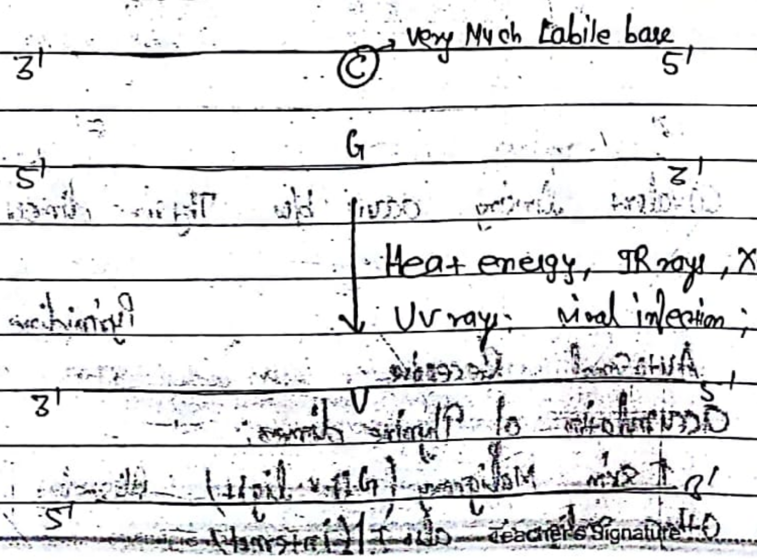
∴ Cutting Phosphodiester bond

→ Enzymes involved → Exinuclease enzyme

DNA Polymerase I

DNA ligase (less cases of XP)

BASE EXCISION REPAIR



Heat energy, IR rays, X-ray —

UV rays; viral infection; chemical agents

U ← Uracil DNA glycosylase
↳ Removal of base

3' ——— [U] ——— 5'
G
5' ——— 3'

↓ Nuclease [AP endonuclease] → Apurinic/apyrimidinic
↳ create a nick on either side of defect

3' ——— 5'
G
5' ——— 3'

↓ DNA Polymerase I + DNA ligase

3' ——— C ——— 5'
G
5' ——— 3'

→ Most labile base to change → Cytosine
↓
2-oxy 4 amino pyrimidine

Changes to Uracil → 2,4-dioxy pyrimidine
addition of 1-oxy

Cytosine deamination → Uracil
↳ 4 amino

• AP site [apurinic site or apyrimidinic site] → No base but phosphodiester bond is present.
• AP site is identified by → AP endonuclease
• No known disease due to its effect

Teacher's Signature

Date
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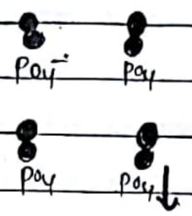
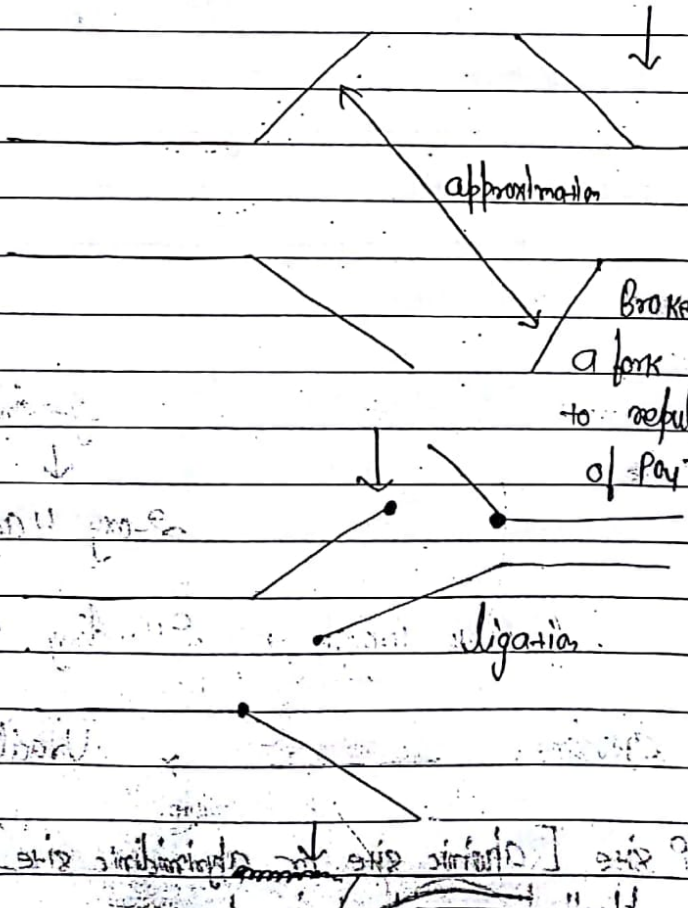
Double-Strand break Repair process



~~Ku protein~~ → Binds c̄ 4 broken ends of DNA
→ Recruits DNA Protein Kinase enzyme
↓
adds ~~Phosphate (Po4) group at broken ends~~



Degraded

Purpose → Repair of Broken ends
– Rearrangement of Nucleotides

NEET'16 This repair is seen in Immunoglobulin gene rearrangement. eg ⇒ Ataxia Telangiectasia[QQ], Bloom's Syndrome, Fanconi's Anemia.

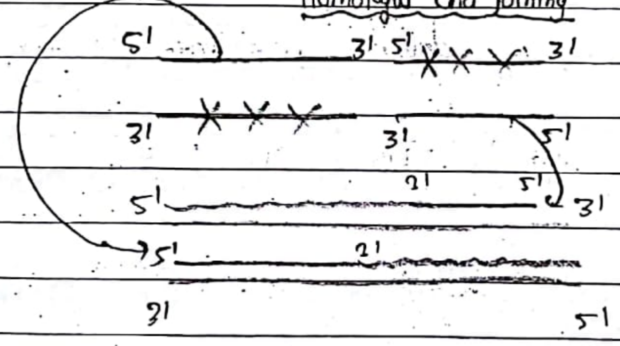
→ Double strand break → Result in genetic Recombination;
↓ Lead to
Chromosomal translocation; Broken chromosome & finally cell death.

(BB) Homologus End joining (Less common) (Less common)

(BB) Non homologus end joining (More common)
↳ Error prone.

AIIMS Nov'17 SCID (Severe Combined Immunodeficiency Disease) ⇒ d/t defective Non Homologus End joining Repair (NHEJ)

Homologus end joining



Teacher's Signature ....

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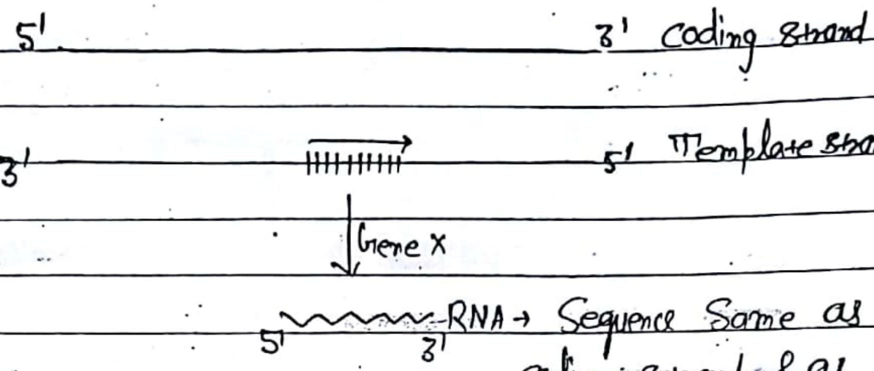
TRANSCRIPTION

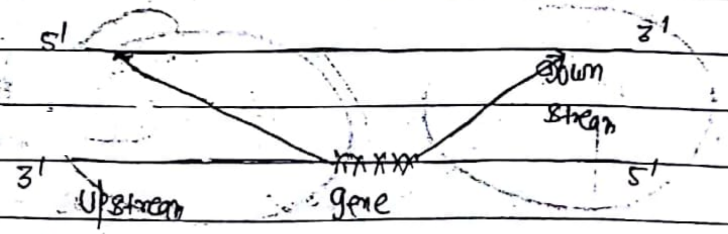
→ Initiation
→ elongation
→ Termination.



- Template strand → produces working copies of RNA molecule
  ↳ also k/as "Non coding / Minus strand / Bottom strand / Antisense"
- coding strand → doesn't participate in transcription.
  ↳ also k/as "Sense / plus / up strand."

Transcriptional control elements

- Portion of template strand towards 5'-end of coding strand → Up stream portion
  from 1st Nucleotide of gene
- Portion of template strand after gene towards 3'-end of coding strand → Down stream portion



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Date
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- Binding of Transcription Regulatory proteins to DNA is Regulated by →
  - i) Helix-turn Helix Motifs;] → Symmetrical Palindromes
  - ii) Leucine-Zipper Motifs; ← Periodic Repeat of Leucine Residue at every 7th Position
  - iii) Zinc finger Motifs (For Steroid Receptor Family & Thyroid Receptor Family)

Binding site is Repeated by 2-9 times

TCE - Transcriptional control Element → Req for regulation of expression (+ or -)

Basal control elements → Always upstream

Regulatory elements → May be upstream or downstream

Element = Segment of DNA

Promoter proximal element → "CAAT" box or "GC" box (-75 position) Eukaryotes

Promoter area or element ↓ decides the location where RNA Polymerase binds the DNA

→ -35 box → 5' TTGA 3' Prokaryotes

- decides the frequency of basal transcription.

* central dogma of genetics do have amplification.

DNA (one) → RNA (Multiple) No. of RNA → Protein (1 RNA Made very protein)

Promotor proximal area

3' ← upstream → Binding of RNA Polymerase — Gene 5'

Promoter Region → +1 Nucleotide (In downward direction "+") ; -1 Nucleotide (In upward stream direction "-")

↓

Rich in TA

Prokaryote ↓ Pribnow box (5' TATAAT 3') (-10 Position)

Eukaryote "Goldberg Hogness box" ↓ "TATA" Box / Hogness Box (-25 Nucleotide upstream) (TATAAA sequence)

Teacher's Signature ....

Promoter proximal element → decides the duration of binding of RNA Polymerase to template strand

↓

decides frequency of transcription

if RNA Polymerase binds to template strand for more duration → Many gene expressed

↓

More RNA Produced

↓

More protein

↓

K/as "Amplification of central dogma"

Regulatory elements

Located far from genes.

↓

K/as "Distal regulatory elements".

- Enhancers
- Repressors
- Hormones Binding element
- Toxin Binding element

} Bind & change or alter the expression

~~Replication~~ } ~~In nucleus~~
~~Transcription~~

RNA Polymerase → ~~5'-3' Polymerase activity only~~

Teacher's Signature ....

Termination of RNA Synthesis :-

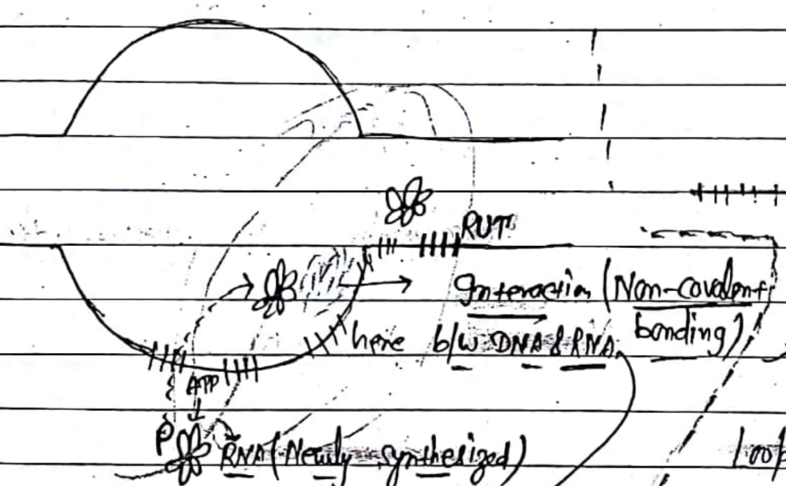
In Prokaryotes	In Eukaryotes
(1) ρ-dependent termination	Ill defined
(2) ρ-Independent termination	

ρ (Rho) dependent

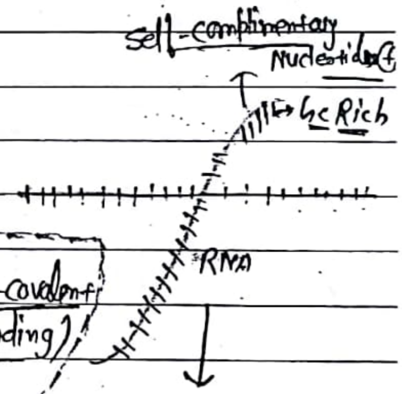
Requires →
- ρ factor
- ATP
- RUT element at Gene end
  ↓
  Rho utilizing termination signal

ρ (Rho) Independent

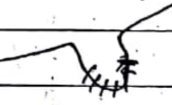
- None of 3 things is required.
- requires specific sequence of RNA (GC Rich).



Stabilize RNA in the bubble



Loop formed at GC end



- Knot "Lariat or Hairpin loop or stem loop"
- No ATP Required
- Loop formed due to H bond b/w complimentary bases

ρ factor → Hexameric protein
→ Captures RNA & ascend on this RNA
→ 6 Subunits
→ Uses ATP
→ Reaches extreme end of RNA
→ After identifying RUT element enzymatic activity is induced in ρ factor

↓

DNA-RNA helicase

↓

Interaction b/w DNA RNA is lost

↓

RNA free from bubble

↓

Termination of transcription.

→ Not identified in Eukaryotes

→ Types of RNA & Post transcriptional modification's

In Eukaryote

Gene	Enzyme	Product
I	RNA Polymerase I	In Nucleolus formation; 45S rRNAs
II	RNA Polymerase II	mRNA; miRNA; snRNA (micro)
III	RNA Polymerase III	tRNA; 5S rRNA; U6 snRNA

Mnemonic :- RMT

↱ 5'–3' Polymerase Activity (No 3'–5' exonuclease activity)

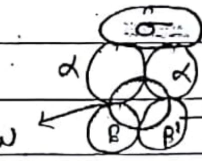
In Prokaryote → ~~RNA Polymerase~~ → K/a "~~core enzyme~~" → has complete ~~transcribing~~ ability; May not form correct RNA

components: 2α, 1β & $1\beta'$, 1ω

· Rifampicin inhibits β-subunit of RNA Polymerase

· For correct RNA Synthesis in Prokaryotes σ (sigma factor) is required.

σ factor + Core Enzyme → Holoenzyme → Catalytically active
↳ Resides in β subunit.



σ factor binds to promotor area.
↳ ∴ correct binding is due to σ factor

rRNA → Post transcriptional Modification of 45s rRNA → Cleavage in Nucleolus.

Prokaryotic
· 5s rRNA
· 16s rRNA
· 23s rRNA

Eukaryotic
· 5s rRNA — In nucleus — RNA Polymerase III — Independently synthesized
In Nucleolus:
· 5.8s rRNA
· 18s rRNA
· 28s rRNA
Synthesized as rRNA Precursor ↓ by RNA Polymerase I

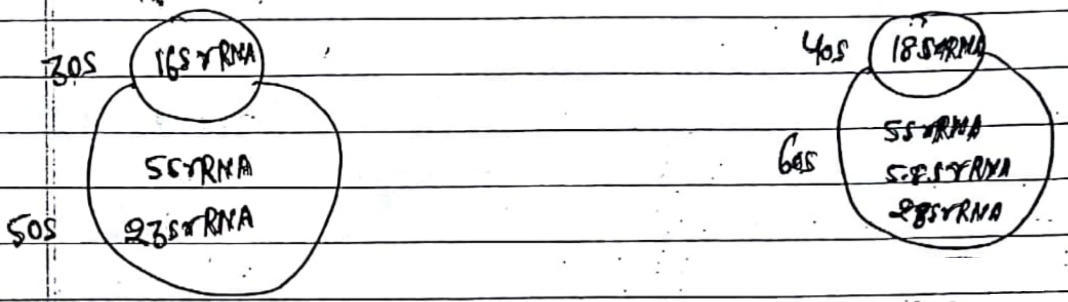
~~Svedberg Unit~~
↳ Sedimentation Rate.

· Most abundant RNA in cell → rRNA

85% ⇒ rRNA
10-15% ⇒ tRNA
~5% ⇒ mRNA

Function → Making structure of Ribosome
↓
rRNA + Protein

Ribosome
→ Prokaryotic
→ Eukaryotic



70s ribosome

80s Ribosome

23s rRNA
28s rRNA
→ It is a Ribozyme → RNA: showing catalytic activity
↓
Peptidyl transferase activity
↓
Present in large subunit of Ribosome

rRNA → formed in Nucleolus***
→ cleaved into smaller units (Post transcriptional modification)
↓
5.8s, 18s, 28s

↓
occurs in Nucleolus

- Codon → Unambiguous → Codes for single AA.
- Poly "A" tail translates into "POLYLYSINE". QQ

Date ____ Page 131 (67)

mRNA → Eukaryote / Prokaryote

Prokaryote → Start translational quickly

Eukaryote:

HnRNA → Heteronuclear / Heterogenous RNA / 1° transcript
↓ Precursor of mRNA

HnRNA ↓ mRNA

HnRNA → Very large molecule as compared to mRNA.

→ Post transcriptional modification of HnRNA (in Nucleus only) :-

5' E | I | E | I | E | I | E 3'
HnRNA

Nucleus

Exons ⇒ coding (expressed sequence)
Introns ⇒ Non-coding (Non-Expressed Sequence)

- Inside Nucleus
- Capping at 5' end (7 methyl Guanosine triphosphate)
- Splicing (Removal of introns)
- Tailing at 3' end (Poly A Tail) ⇓ 80-250 AA.
  ↳ Never transcribed.

* Capping & Tailing is for protection of m-RNA from exonuclease

5' E | E | E | E | E or little intron 3'
mRNA
↳ Seen towards ends, if part. [all introns are not removed]

↳ Much shorter
↳ Comes out of Nucleus → to cytosol

Splicing → by Spliceosome → Remove Intron / joins exons
⇓
Components → ① Intron
② SnRNA (Small Nuclear RNA)
③ SnRNP

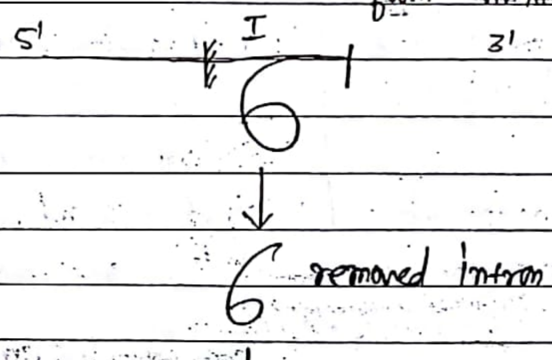
Teacher's Signature ....

SnRP → Small nuclear Ribosomal proteins
↳ Function → Positioning the spliceosome on introns

Types of SnRNA → U_1, U_2, U_4, U_5, U_6

U_1	U_4	U_6
U_2	U_5	

SnRNA is a Ribozyme
↳ Phosphodiesterase activity
↓
Cut the intron at 5' end & then intron loops around itself & removed from mRNA.



↓
automatic loop formation.

Faulty splicing → Removal of wrong intron by mistake
↓
this formed mRNA will not be translated.

Eg → β-thalassemia,
Spinal Muscular dystrophy.

Teacher's Signature ....

- only about 1.5% of human DNA is coding (exon DNA), carrying information for protein products.
- Post-transcriptional Modification of RNA → i) 5'-capping; ii) Addition of Poly-A tail (Polyadenylation); iii) Removal of introns (Splicing).

$\boxed{\text{Alternate Splicing}}$ → Physiological process

1 gene → Results in >1 types of Protein.
(generally 1 gene — 1 type of protein)

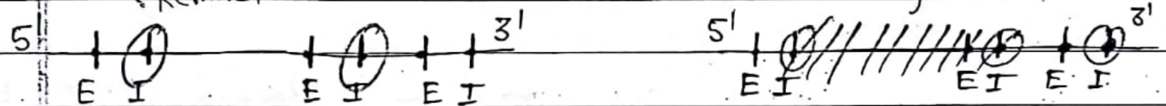
Gene X

on Day 1 ↓ formed hnRNA

on D10 ↓ formed hnRNA → exactly same

Removed intron

degraded



Fusion b/w Nearby Exons ↓

Fusion b/w far away exons ↓

5' ——— 3' mRNA (long)

5' ——— 3' mRNA (short) → Not Same

A process by which same gene results in formation of different mRNA at different time frame due to joining of adjacent exons at one moment of time & joining of far away exons at different time frame.

⇓

Different mRNA → hence diff. Proteins

eg → Formation of different types of Immunoglobulin from the same chain.

- $\boxed{\text{Self-splicing}}$ ⇒ occurs for Rare introns that form a Ribozyme, perform the functions of spliceosome by RNA alone.
  ↳ "Lariat intermediates" are formed.

Teacher's Signature ....

occurs in "Ribosomes"

TRANSLATION

→ Biosynthesis of a protein or a polypeptide in a living cell.

→ Stages of translation :-

i) Requirement of the components;

ii) Activation of amino acids;

iii) Protein Synthesis Proper;

iv) Chaperones & protein folding;

v) Post translational modifications

→ "Aminoacyl tRNA Synthetase" - enzyme works here

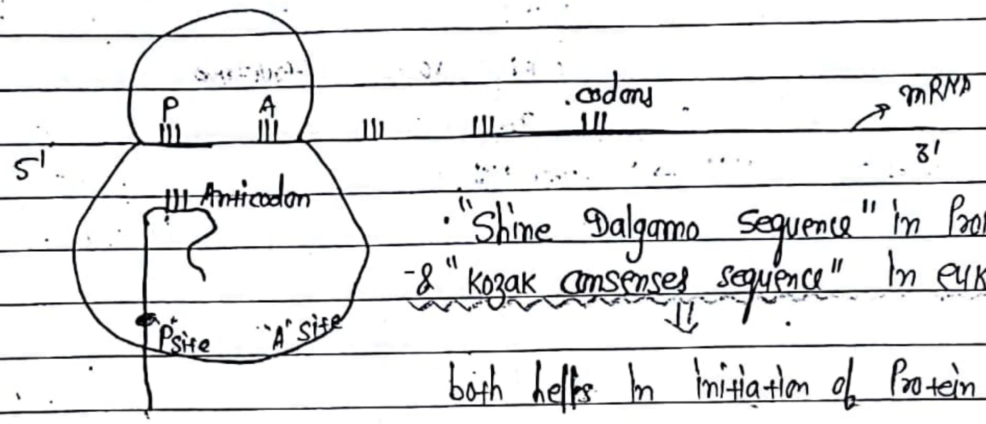
Protein Synthesis Proper

Prokaryotic mRNAs → Polycistronic
↳ a single mRNA has many coding regions that code for different polypeptide

Eukaryotic mRNAs → Monocistronic
↳ code for single polypeptide

→ mRNA binds to Smaller Subunit of Ribosomes;
↳ it read in the 5'-3' dirn & the polypeptide synthesis proceeds from N-terminal end to C-terminal end

Teacher's Signature



- "Shine Dalgarno sequence" in Prokaryotes & "Kozak consenses sequence" in eukaryotes
  ⇓
  both helps in initiation of Protein synthesis.

m tRNA
↳ Methionine → ~~only AA who binds directly "P" site of large subunit of Ribosome~~

80S initiation complex

IF → Initiation factor

(Prevent premature Reassociation of 60S & 40S)
IF-3
IF-1A

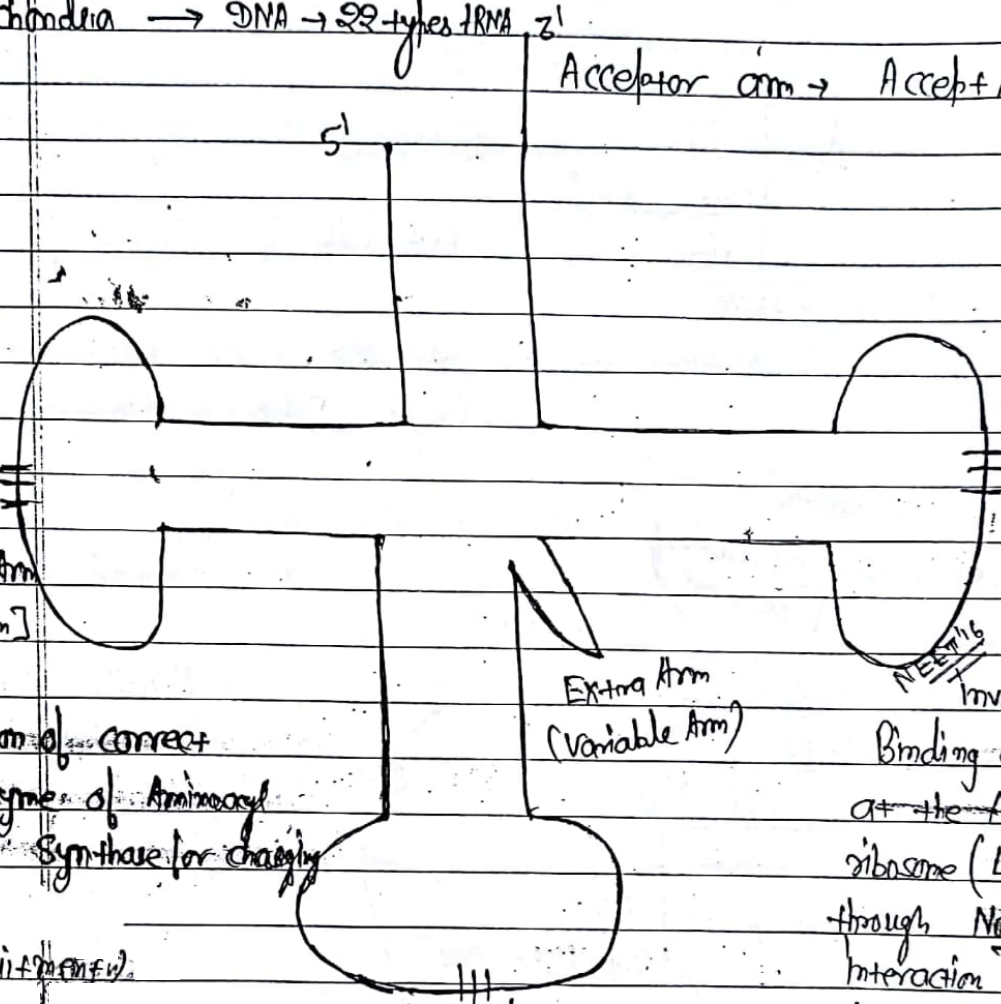
80S → 40S, 60S

- ~~Small subunit of Ribosome undergoes modification~~
- ~~Large subunit of Ribosome doesn't undergoes any Modification~~

40S
↓ IF2, GTP, "methionine tRNA"
43S PIC ← 43S preinitiation complex
↓ IF 4F (4A subunit, 4G, 4E), mRNA, ATP
48S IC — 48S initiation complex
48S IC + 60S → (IF-5) → 80S initiation complex

$\boxed{\text{tRNA}}$ → 31 types for Nuclear DNA; for 20 AA
- Clover leaf structure;
- 5 arms

Mitochondria → DNA → 22 types tRNA



- Carry one AA, we have more than one tRNA for one AA
  eg → Serine → 6 tRNA

→ Charging of AA on tRNA → Should be correct
↓ done by Aminoacyl tRNA Synthase enzyme
↓ In order to form correct Protein.

Q. 1 peptide bond formation Needs Four high energy phosphates.

* P & A site ⇒ canonical site

Aminoacyl tRNA Synthase → function → charging of correct AA on tRNA, matching with its anticodon.

→ Cytosolic enzyme
20 iso-enzyme;
Proof reading activity. AIIMS-91

charging of t-RNA

- Activation of AA
  ↓
  ATP used
- Transfer of activated AA to 3' end of tRNA

Eg → Glycine —(ATP → AMP)→ Glycyl
↓
active AA
↓
2 high energy phosphate is used

- t RNA loading c̄ AA → goes to A site
  ∴ New tRNA attaches to Acceptor site (A-site)
  1st tRNA → P site
  ↳ Methionine

→ 1st peptide bond formed at A site.

Teacher's Signature.........................

Date
Page 138

* Methionine Make "N" terminal of protein.
Amino N — C (COOH)

* Synthesis of Protein ⇒ "N" terminal to "C" (NB) terminal of protein

Peptidyl transferase →

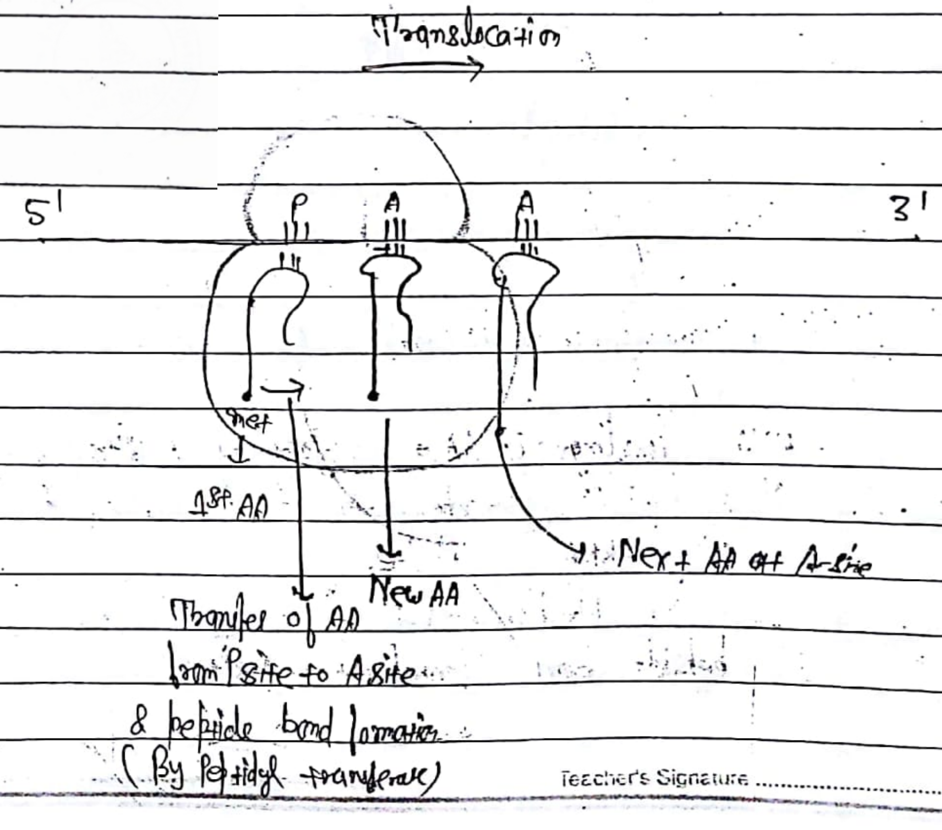
Transfer AA from P site to A site
↓
Peptide bond is formed.

Translocation → GTP used (GTP → GDP)

→ Ribosome moves on mRNA

→ New site will became A site & Previous A site becomes P site

Protein synthesis is a cyclical process.



Teacher's Signature ....

Formation of a Peptide Bond → (4) Pi (high energy phosphate used)

(1) ATP → AMP (charging) → (2) Pi

(2) GTP → GDP (Translocation) → (1) Pi

(3) GTP → GDP (either Peptidyl transferase activity or localization of tRNA at A site)

Termination → By stop codon at A site
- UAA → Ochre
- UAG → Amber
- UGA → opal

- Releasing factor bind at A site [RF]; when stop codon is encountered

- RF ē the help of
  - H_2O**
  - Peptidyl transferase**
  - GTP**

  Release Polypeptide from P-site

A site → will have stop codons

∴ Protein is released from P-site

RF → also dissociates Ribosome to Small & large Subunit

⇒ Small Subunit undergoes modification & different Ribosome is formed

Teacher's Signature ....

Date
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• Fidelity (faithfullness) of protein synthesis
↳ according to codon on mRNA the AA sequence should be same

Maintained by — ① ~~D Amn~~
② Aminoacyl tRNA synthase

• Maximum modified bases ⟶ tRNA

Pseudouridine
dihydrouridine
IMP
Hypoxanthine etc

→ 2006 Noble Prize given for its discovery QQ

Small RNA → miRNA (Micro)
↳ Regulatory RNA
↓
Causes gene suppression

* Drosha cut & form Pre mi RNA & (Pre mi RNA) goes outside Nucleus & Form "RISC"

→ 22-25 nucleotide, ssRNA

PremiRNA ⇒ double standard

RISC ⇒ SSRNA → Part of RISC in cytosol
miRNA ⇒ SSRNA
↓
RNA Induced silencing complex

miRNA
↓ cut by Drosha
PremiRNA
↓
RIS

• MiRNA once if bind to mRNA → causes degradation of that mRNA
• Transcribed by "RNA Polymerase II & Drosha". QQ
II enzyme involved in cutting process

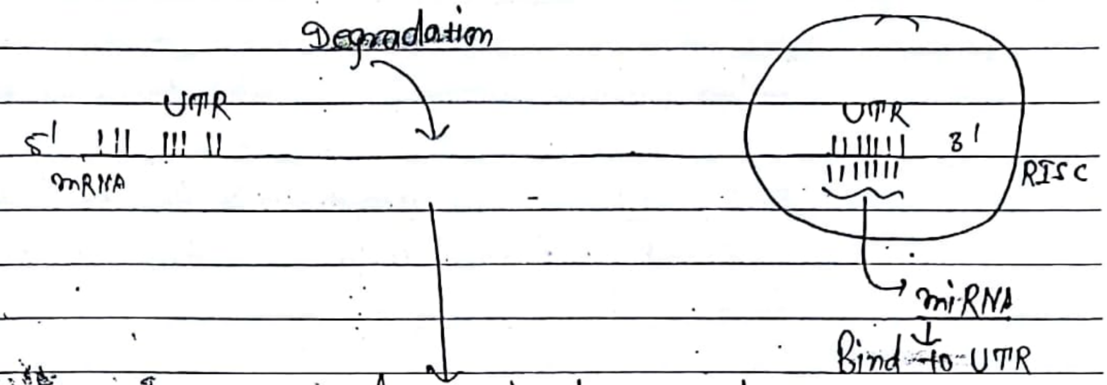
Teacher's Signature ....

"p" Bodies ⇒ found in cytoplasm; Contains Nuclease.

* Lack of miRNA ⇒ d/w Malignancy;

* miRNA ⇒ (N) cells.

UTR ⇒ Untranslated Region.



No translation in presence of miRNA

→ mRNA in presence of miRNA will not form protein.

Small Interfering RNA / silencing RNA

SiRNA → Exogenous miRNA

→ Used for RNA Interference / RNA Silencing.

→ experimental use to suppress disease causing mRNA translation

→ double stranded (22-25 Nucleotide Long)

→ Size same as miRNA

→ Binds anywhere along mRNA; not necessarily on UTR.

→ Gene suppression by degrading mRNA (function same as miRNA)

Teacher's Signature ....

Date
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→ gene of one species is incorporated in vector so as to produce Recombinent vector, so, as to utilize Various Purposes

Recombinent DNA technology or genetic Engineering or DNA cloning

Loading of vector c Recombinent DNA

DNA library → Various Nucleotide sequence is stored which represent the genomic constituent of one species

2 types — genomic
— Complimentry

For Sequence analysis → Genomic DNA library → eg → Polymorphism study

For Protein Synthesis → Complimentary DNA library

Genomic DNA library:
↓
Nuclear DNA stored
↓
Gene may be expresed or Unexpressed
↓
It has to undergo transcription
↓
mRNA formed → Protein may or may not form

Complimentary DNA library:
↓ cDNA
DNA Complimentry to mRNA is stored
[i.e Reverse transcriptase copy of mRNA]
↓
Only expresed genes (+)
↓
Protein will form

Size of gene → Large | Small

In both library Recombinant tech is used to store DNA c̄ vector

Teacher's Signature ....

→ Plasmid artificial chromosome

Vectors ÷ Size of DNA that can accomodated in the vector:

Vector		DNA insert size (kb) (Amount of DNA accomodated in vector)
Natural	Plasmid PBR322 →	0.01 – 10 Kb
	Lambda charon 4A (Bacteriophage) →	10 – 20 Kb
Artificial	Cosmids →	35 – 50 kb
	BAC (Bacterial Artificial Chromosome) →	50 – 200 Kb
	PAC →	100 – 300 kb
	YAC →	500 – 3000 kb

↳ Yeast artificial chromosome

$\boxed{\text{Restriction Endonuclease}}$ →

→ Cut both strands of DNA together
→ K/as "Molecular Scissor".
→ Extracted from Bacteria
↓
effect of bacteriophage on bacteria is 'restricted' from this enzyme
↓
bacteria is saved & Phage degraded
↓
So K/as "Restriction Enzyme".

→ It acts on Palindromes

Palindromes [2-6 Nucleotides] ⇒ Regional areas of double standard DNA

```
     ───→
5'  A C A | G A T C | C G A C C  3'
3'  T G T | C T A G | G C T G G  5'
     ←───
```

Sequence Same in both strands in 5'→3' dirn which are showing symmetry of Nucleotide sequence of either strand of the DNA, when they are being read in particular direction

Teacher's Signature

Date
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ends formed after action of RE

↳ Sticky or staggery or overhanging ends
↓
due to complimentary bases they can reattach.

↳ Blunt ends may also form.

BLOTTING TECHNOLOGY

↳ To detect a specific segment of the DNA in whole genome.

(1) Southern blotting → invitro DNA hybridization
↳ for DNA
→ to Search particular Sequence of nucleotide in DNA (eg → Mutation Sequence)
↳ Qualitative Analysis.

Steps to follow :-
- DNA extraction
- RE (Restriction Endonuclease)
- Electrophoresis
- Denaturation of DNA using alkali
- Blotting over nitrocellulose paper
- Fixing at 80°C temp for 1hr
- Layering ō Radioactive Probe
- Washing
- AutoRadiography to detect radioactive Probe.

Technology → Prepare probe → Complimentary to sequence to be Searched
↳ Single strand Sequence

Teacher's Signature ....

RE is used to fragment DNA.

Electrophoresis on Agarose gel

↓

Separation of DNA fragment

↓

Neutral particle can't move

criteria of mobility → Charge : Mass ratio.

Whole gel with DNA is transfered to weak alkali
1/2 N NaOH

↓

Denaturation

Blotting on Nitrocellulose paper (Not visible)

↓

DNA fragments are transfered on this

↓

Heat fixation (80°C for 1 hr)

↓

Probe layering

allow hybridize by washing

"cumblesome procedure"

[Probe is labelled with
fluorescent Material
enzyme (calese)
Radioactive labelling]
↳ Hazardous

↳ enzyme convert → colourless substrate to colored product

Washing is done to remove unbound Probe

↓

Examine the Probe

Probe is used to hybridize the desired segment

Gene Expression in Real time 146
↳ mRNA More → More gene expression

NORTHERN BLOTTING ⇒ Quantitative technique

Gene X
↓
mRNA
↓
Complimentary DNA
↓
Probe
↳ complimentary to cDNA.

→ Technique for the specific identification of RNA molecules.

Blotting done by using a chemically reactive paper prepared by → diazotization of Aminobenzyl-oxymethyl to create diazobenzyloxymethyl (DBM) paper

DOT- BLOTTING

· Technique by which the nucleic acids (RNA or DNA) are directly spotted onto the filter
↓
Not subjected to electrophoresis

WESTERN BLOTTING ⇒ detect Ag-Ab.

→ It involves identification of proteins.

→ Very useful to understand Nucleic acid function

→ Single phenotype; Single locus & Multiple Normal allele.

POLYMORPHISM

- Change of Nucleotide sequence at the level of DNA in human population.

Short (2-6 bp) Tandem Repeats, Less than 1 Kb

Types → SNP, VNTR

VNTR → MicroSatellite, Minisatellite (15-70 base pair & 1-3 Kb.)

SNP — Single nucleotide Polymorphism / We Need Probe

VNTR — don't Need Probe. Variable No. of Tandem Repeat

eg → ABCABCABC / 121212

(Tandem ⇒ one after Another)

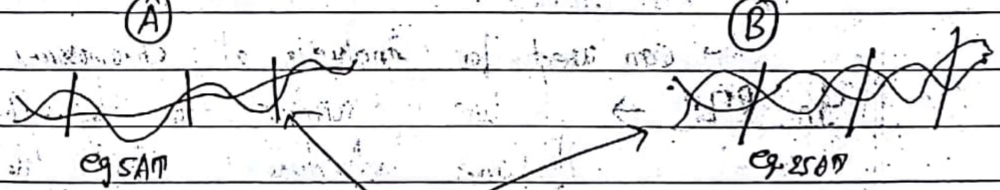
eg →

Ⓐ	Ⓑ	Ⓐ	Ⓑ
CACCGATC	CACCGAAC	ACATATCG	ACATATATAT
		② AT	④ AT

- May or may not change Palindrome
- SNP will not change length of DNA

VNTR also changes length of DNA

Q. Compare sample A & sample B of DNA & find out if they belong to same person !→



Suppose in 2nd segment we have VNTR polymorphism
↓
So length changes

RE → Same RE used for both A&B so same Palindromes are targeted

↓

take the fragment in the well & go for electrophoresis

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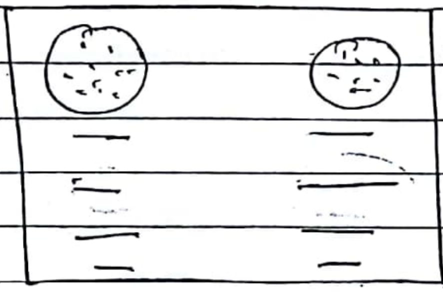
~~RFLP:- Restriction fragment length Polymorphism~~
↳ Single base pair substitutions in germ line DNA of different Individuals that either destroys or ~~creates new recognition site for a given restriction enzyme~~



→ We get bands of DNA
↓
Band Pattern if not Same for both DNA

~~Ethidium Bromide~~ is used to see these bands under UV light
↓
Bands appear orange colour

Bands if Same → DNA of Same person

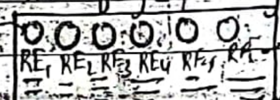
Bands if different → DNA of different person.
↓
due to VNTR

→ Fragment, if different in length, will move differently on electrophoresis (charge : Mass ratio)
→ can used for Analysis of chromosomes structures. QQ

In RFLP → We examine the Polymorphism
↳ if same Individual → Same Polymorphism
↳ detects variation in DNA sequence by Southern blotting. QQ

eg. → crime Investigation
Paternity disputes

Principle of DNA fingerprinting → Polymorphism
↓
VNTR



different Restriction endonuclease

Chromosome walking → It will eventually define the disease locus.

Teacher's Signature

Kary Mullis (1993) → Noble Prize

PCR → Amplification of DNA Segment.

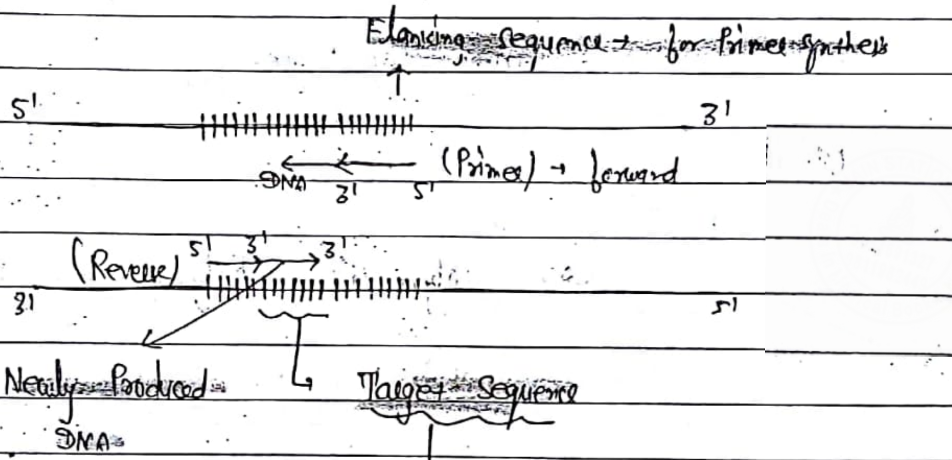
Steps :- (1) Primer construction;

(2) DNA denaturation;

(3) Primer annealing.

(4) extension of Primer with DNA Polymerase.

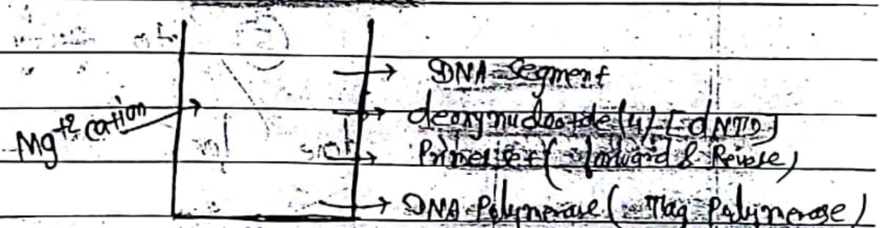
- PCR is based on Principal of Natural DNA Replication



→ which needs to be amplified

- For 1 DNA PCR → 2 Primer are required.
- For PCR → Flanking sequence is must to know
  → Target sequence is not must

Tube used → PCR cuvette **



↓ Thermus aquaticus

- Place this cuvette in PCR machine.
- Each cycle
  ↓

① 92–96°c [94°c] → for 10 min
↓
Denaturation of DNA

② 45°c for 4 min
↓
Annealing of Primer

PCR
↓
Cyclical Rxn

③ 72°c for variable time
↓
DNA Polymerisation (elongation)

72°c → Optimum temp for Taq Polymerase
↓
Same sample is repeated to get multiple sample of same target DNA

Clinical application →
① In FMT
② to detect infectious agt.
③ Prenatal genetic diagnosis
④ tissue typing for transplants
⑤ to study evolution

PCR can be done for DNA, RNA
↓ ↓
PCR RT-PCR

RTPCR → Reverse transcriptase PCR

→ RNA
→ Tth Polymerase (Thermus thermophillus)
↳ having additional DNA Polymerase Reverse transcriptase activity.

Mg^{2+}

Primer

dNTPs (4)

Real time PCR (qPCR → quantitative)

PCR product can be quantified at any moment by looking at the amount of fluorescence

Can be done for DNA as well as RNA.

→ DNA
→ DNA Polymerase
→ dNTPs (4)
→ Dye (Real time Dye) → fluorescent dye
- SYBR green dye
- Molecular Beacon dye
- Taq man dye

Mg^{2+}

M/C & most non-toxic dye → SYBR green dye. → Intercalate the DNA
(डीएनए में इन्टरकेलेट करता)

In PCR we use DNA Polymerase only once (Not in each cycle) as they are thermostable

Teacher's Signature ....

Date ______
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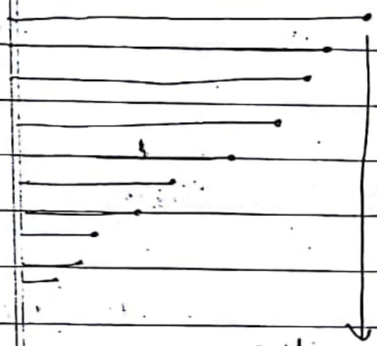
Q. Which is not req. in PCR–

1. dNTP
2. Thermostable enzyme
3. dideoxyribonucleotide → will stop DNA Synthesis abruptly
4. ✓ DNA template

→ was used for DNA sequencing by Sanger
↓
Sanger's enzymatic DNA sequencing
dideoxynucleotide trail sequencing
↓
Can also be used to detect the mutation

DNA



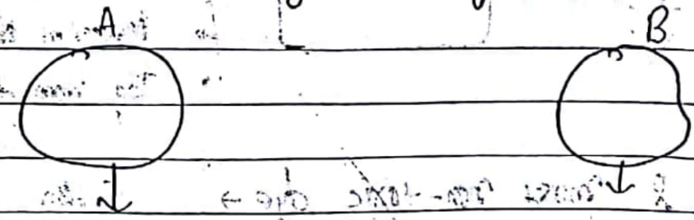
Replication stopped by use of ddNTP.

MICROARRAY (DNA CHIP)

also known as "Comparative genomic hybridisation (CGH)".

Principle → Hybridization

Compare the genome of two cells →

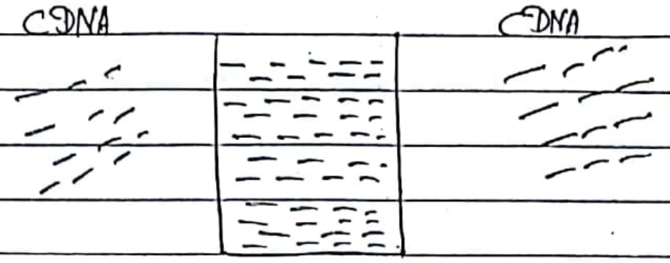


Isolate mRNA ↓ mRNA ↓

Teacher's Signature ......

Colour coded

CDNA CDNA



Wells Magnified

Gene

Coated c̄ mRNA
↓
different wells c̄ different mRNA (commercially available)

Well

Micro chip (DNA chip)

Micro wells
↓
Coating of Nucleotide Sequence on internal wall
↓
expressed material (seq. of mRNA) is coated on internal wall

↓

all wells are filled with c̄DNA
↓
Let the chip hybridize
↓
Wash (unbound CDNA washed away)
↓
different wells are now showing different colour

Max^m wells → Yellow colour

Red colour → Annealing of CDNA (this gene only in B cell)

Yellow colour → Hybridization
↳ So, Particular gene is present in both cell

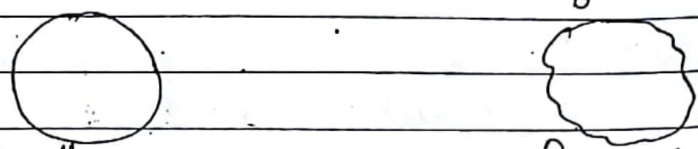
Teacher's Signature ....

Green colour → gene only present in cell A

Black colour → gene not present in any cell

eg →

A B



N cell Tumor cell

↓ ↓

mRNA mRNA

Microarray will tell us → gene (+) in Normal cell but suppressed in tumor cell

→ gene Not expressed in Normal cell but expressed in tumor cell etc

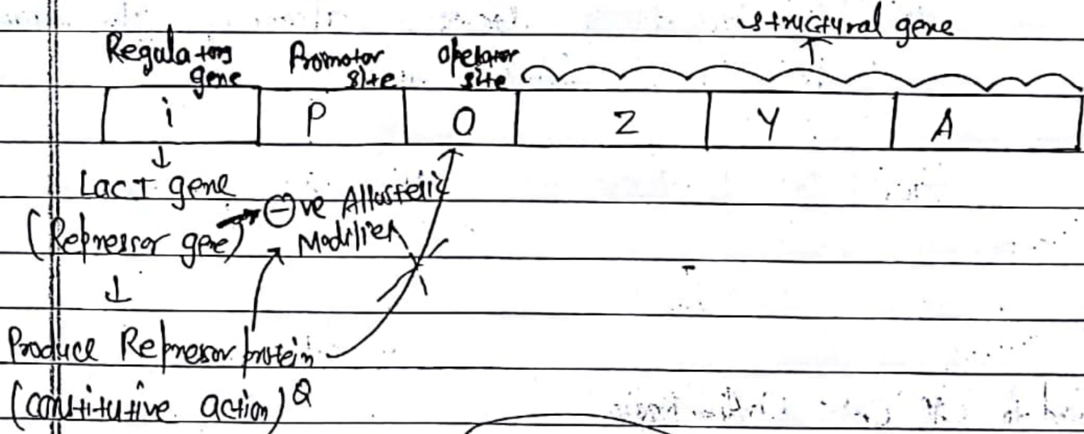
LAC OPERON MODEL

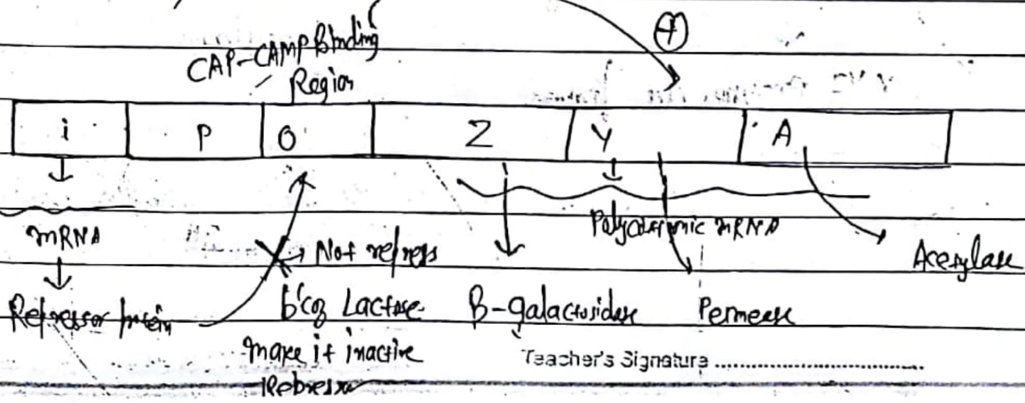
- ~~3 structural genes~~, ~~one operator gene~~, ~~one Promotor site~~ & ~~1 Regulatory gene~~

- Prokaryotic gene regulation model by Jacob & Monod.

- He explained why Lactobacillus is able to form enzyme of Lactose catabolism only in presence of Lactose and absense of glucose

Lactobacillus
↓ forms
enzyme of lactose catabolism
↙ only if
Lactose ⊕
glucose ⊖





Teacher's Signature ....

- Lac I gene bind to operator gene
- RNA Polymerase bind to promotor area but not able to move further b/c operator is not free
- Operator becomes free → in presence of Lactose

Lactose —(+)→ Allolactose → Induces
↓
Inhibit binding of Repressor protein to operator Area.

If glucose is present CAP-CAMP protein is not formed
↓
So, Lack of glucose is also must. QQ Positive allosteric modifier of Lac operon.

So, in presence of lactose & absence of glucose enzyme synthesis by Lactobacillus occurs.

- Lack of glucose → CAP (Protein) – CAMP formation
↓
Transcription
↓
Translation

Bind to CAP-CAMP Binding Region
↓
X Y Z enzymes are formed

CAP ⇒ Catabolite Activator Protein
↳ Positive Regulator QQ

Hb → (4) globin + (4) Heme (Prosthetic group)

Date
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(80)

• one gram of Hb contains 3.34 mg of iron

HEME SYNTHESIS

+ See Page 230 & 231

• In Bone marrow (85%) ↓ No Regulation ↓ ALA II enzyme

& Liver (15%) ↓ Regulated Pathway ↓ ALA I Synthase ↓ Q Rate limiting enzyme

• Partly mitochondrial partly cytosolic

• Precursor → Glycine & Succinyl choline

	Deficiency		Condition
Hepatic Porphyria ↑ (1)	PBG (Porphobilinogen) Deaminase / UPG – I Synthase	→	(AIP) Acute Intermittent Porphyria
Erythropoietic Porphyria ↑ (2)	UPG (Uroporphyrinogen) III Synthase	→	(CEP) Congenital erythropoietic porphyria
Hepatic Porphyria ↑ (3)	UPG III Decarboxylase	→	Porphyria cutanea tarda (M/c Porphyria)
Hepatic Porphyria ↑ (4)	CPG (coproporphyrinogen) oxidase	→	Hereditary coproporphyria ↳ also cause "Harderoporphyria" (erythropoietic Porphyria)
Hepatic Porphyria ↑ (5)	PPG (Protoporphyrinogen) oxidase	→	Variegate Porphyria
Erythropoietic Porphyria ↑ (6)	Ferrochelatase	→	Protoporphyria

* Soret bands → Sharp Absorption band near 400 nm; Shared by all Porphyrins.

• Porphyrinogens are colorless; while the porphyrins are colored.

Teacher's Signature ....

* Depression of ALA Synthase :⇒ biochemical basis of precipitation of Porphyria by barbiturates

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Heme

(Liver)

Mitochondria

Glycine + Succinyl CoA

↓ ALA Synthase (PLP) NEET'16 ⊖ (Heme)

α-amino β keto adipic acid

↓ ALAS

Amino levulinic acid

Cytosol

2 ALA

↓ ALA dehydratase / PBG synthase ($2H_2O$)

4 PBG

↓ PBG Deaminase / HMB synthase or UPG I Synthase ($4NH_3$)

HMB (Hydroxy Methyl Bilane)

↓ UPG III Synthase

UPG III

↓ UPG III Decarboxylase

CPG III

↓ CPG III oxidase

PPG IX

↓ PPG oxidase

Protoporphyrin IX

↓ Ferrochelatase (Fe^{+2})

Heme

↳ Spectrophotometry can be used to detect Porphyrins & their precursor in Porphyria

PORPHYRIAS

Characterized by increased excretion of porphyrins or porphyrin precursors.

Porphyria → Cutaneous Sign & Symptom
Porphyria → Neurological Sign & Symptom

Teacher's Signature ....

Date
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(81)

- ~~Ehrlich's aldehyde test~~ + ~~Porphobilinogen & urobilinogen in Urine~~
- Uroporphyrin ⇒ Most water-soluble of the porphyrin; excreted in urine
- Protoporphyrin ⇒ Least water-soluble; so; excreted only in bile

Purely Neurological	Purely cutaneous	Both
• Acute intermittent Porphyria	• Congenital erythropoietic Porphyria	• Hereditary Coproporphyria
	• Porphyria cutanea tarda	• Variegate Porphyria
	• Protoporphyria	

- All Porphyria are Autosomal dominant except for Congenital erythropoietic Porphyria → Autosomal Recessive
- "all of the above are "hereditary Porphyria""
- Acquired Porphyria → Plumbo porphyria
  ↓
  NEET'16 Lead affect ALA dehydratase > Ferrochelatase
  ↓
  Purely Neurological
- ALA Synthase deficiency → X-linked sideroblastic Anemia

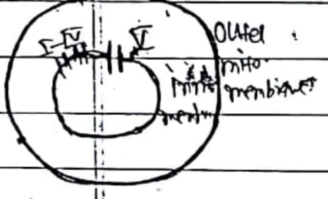
Porphyrinogen	Porphyrins
Reduced Porphyrin	
Colourless	Coloured
X	Luminous
Methylene bridges	Methenyl bridges

Teacher's Signature ....

- Deficiency of complex I, sometimes complex IV ⇒ MELAS (Mitochondrial Encephalopathy with lactic Acidosis)

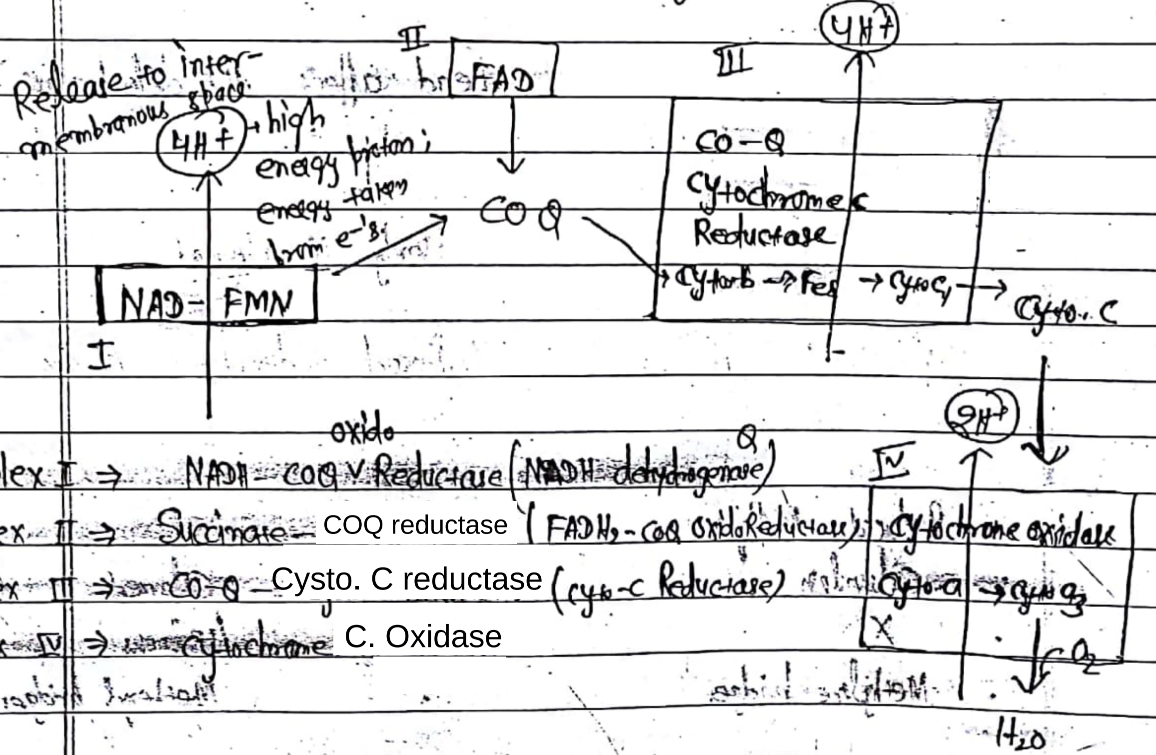
ETC

- Strength of Bond → *** Covalent > Electrostatic > Vanderwall (weakest)
- 5 complexes
- all complexes are present on Inner membrane of Mitochondria.



Complex I – IV → Particle form
Complex V → channel form

- Class-I Enzymes (Oxidoreductase) I – IV → Oxidation
  V ⇒ Phosphorylation
  also known as "ATP Synthase".
  "Oxidative Phosphorylation occur here".



Complex I ⇒ NADH – COQ Reductase (NADH dehydrogenase Q oxido)
Complex II ⇒ Succinate – COQ reductase (FADH2 – COQ OxidoReductase)
Complex III ⇒ CO-Q – Cysto. C reductase (cyto-c Reductase)
Complex IV ⇒ cytochrome C. Oxidase

- Free / Mobile component → COQ, Cyto C
- Last e⁻ acceptor → O_2

→ Reduction potential of K^+ → -2.93

→ NADH (derivative of Nicotinic Acid) & $FADH_2$ (derivative of Riboflavin) Serves as the Substrate for ETC by donating their electrons.

- Criteria for this arrangement → Redox potential

- Redox potential → NAD^+ → $-0.32V$ (min) Means e^- Release easily
  O_2 → $+0.82V$ take e^- easily.

$Fe^{+3}|Fe^{+2}$ → $+0.772$; $Fe^{+3}|Fe^{+2}$ in cytochromes → b → $+0.077$; c_1 → $+0.22$; c → $+0.254$; a → 0.29; a_3 → 0.39

- Redox potential difference across ETC → 1.14 V

- When NADH is starting point → Complex II not encountered.

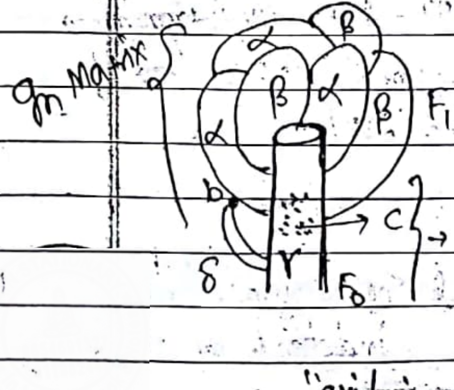
- When $FADH_2$ is starting point → Complex I is not encountered
  → b/c difference of Redox potential b/w CoQ & FAD is very low.

- Complex II → Not accumulating any proton in inter-membranous space.

जैसे-जैसे Complex I → IV तक जाते ; e^- accepted easily & Release energy.

- NADH → $10H^+$ → 2.5 ATP
  $FADH_2$ → $6H^+$ → 1.5 ATP
  4 proton → for formation of one ATP.

Complex V → • ATP Synthase (Multisubunit complex)



γ → Hollow Subunit
3α, 3β, c, δ, b Subunits

c → like a channel along inner membrane

- "Oxidative phosphorylation" occurs at here.

Teacher's Signature ....

Date
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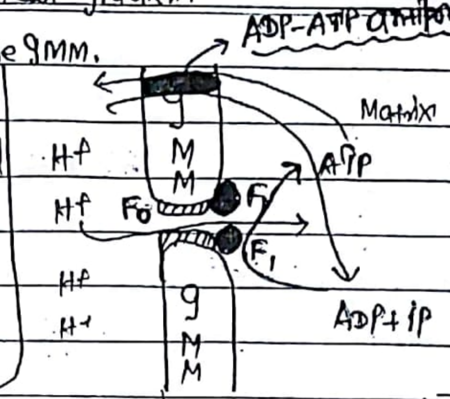
Atractyloside

electro-chemical gradient develops across the IMM.

Outer mitochondrial Membrane



→ oxidative Phosphorylation

* CHEMIOSMOTIC MODEL OF PETER MITCHELL

→ inhibitor of F_0F_1 ATPase in ETC

- movement of H+ is due to proton gradient
  ↓
  rotation of γ Subunit occurs.
  ↓
  Produces catalytic activity in β subunit
  ↓
  ATP synthase activity

[ADP+ip → ATP] ↓ In Matrix

[1β → 1ATP ∴ 3β = 3ATP]
↳ due to 360° of γ → 3ATP formed

Oligomycin QQ
↓
Block the halloomers of γ
↓
Channel block
↓
No ATP Production
↓
∴ oxidation ⊕ Phosphorylation ⊖
↓
In long term oxidation also stop due to ↑↑ H+ in intermembrane space

- Atractyloside → Inhibit ATP-ADP anti-transporter
  ⇒ ATP not formed due to lack of Substrate (ADP).

- UNCOUPLERS → [Both oxidation & Phosphorylation occur independently.]

Teacher's Signature

• RNA Primer Synthesized by → DNA Primase

Date 4/3
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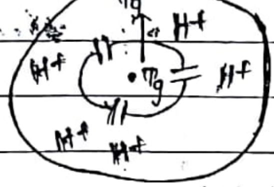
Uncoupler → ~~compound that can uncouple the electron transport from oxidative Phosphorylation~~

by Repeating Protonation & Deprotonation it will do uncoupling

Natural | Chemical

• ~~Thermogenin~~ (Tg) → Permeable across IMM
↓
Protein found in Mitochondria of[Q]
~~Brown adipose cell~~



Tg releases high energy H+ of IM space to low energy H+ of matrix

Tg → Permeable across membrane
↓
Protonation of Thermogenin Heat released
↓
once protonated it goes to matrix
↓
Release H+ in matrix
↓
This proton can't form ATP.

~~ATP only formed via complex V~~

∴ ~~Less Phosphorylation is occuring~~

• 2,4-dinitrophenol
• 2,4-dinitrocresol
↓
damage the inner membrane
↓
Make Perforation (Non-catalytic) in inner membrane
↓
H+ can cross these pores
↓
No ATP formed

• H+ Passing through complex V
↓
ATP formed
⇒ ~~Less Phosphorylation~~

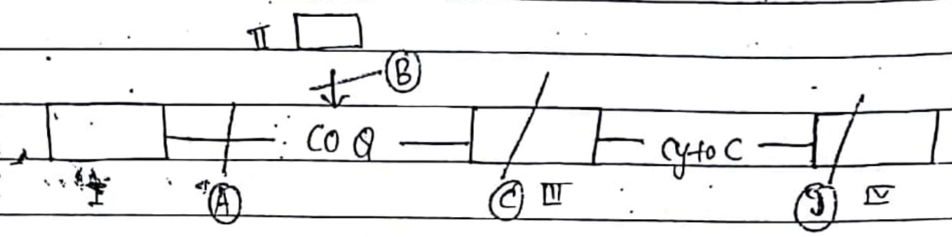
• CCCP ⇒ chloro carbonyl cyano phenyl hydrazone

~~Physiological Uncoupler~~ → ~~Inhibit ATP Synthesis only Not ETC~~
• FFA
• T_3, T_4
• Bilirubin

Teacher's Signature ....

ETC Inhibitors :-

Chemicals which inhibit ETC at same point.



(A) Complex I blocker (b/w I & CoQ)

- Piericidin
- Aminobarbital (Barbiturate)
- Rotenone

$\boxed{\text{PAR}}$

(B) Complex II (b/w II & CoQ)

- Carboxin
- TTFA (Tetrathiofluoroacetate) / Trithionyl fluoroacetate

(C) Complex III

- BAL (British Antilewisite)
- Antimycin A[Q]

**(D) complex IV

Cyanide $\xrightarrow{\ominus}$ cyto-c

- ~~Carbon monoxide~~
- H_2S
- Sodium azide

CN^-

~~CO~~ more toxic

- Reactive oxygen species ⇒ Incomplete Reduction of O_2 & generation of Reactive oxygen Intermediates

↳ also k/a "OFR (oxygen free Radical), ROM (Reactive oxygen Metabolise), AO (Active oxygen)

FREE RADICALS / Reactive oxygen intermediate

- Free Radical are ~~molecule or molecular fragment which are having one or more than one unpaired e^- in the outer orbit.~~

eg → Superoxide $[O_2^-]$

H_2O_2

OH^- (Hydroxyl) → QQ Most potent Free Radical

[O] → Nascent oxygen.

- Source of free Radical →
- Exogenous Agents (CCl_4; ionizing Radiation; cigarette smoking);
- Respiratory Burst
- Electron leakage;
- Enzymatic Rxn;
- ETC (Major Source)
- Viral infection
- UV rays
- Normal oxidation - Reduction Rxn
- Autoxidn of Adrenaline; thiols;
- Ascorbic acid;
- Flavin enzymes
- Enzymes & XO; Aldehyde oxidase; Dihydro-oro dehydrogenase

→ Free Radical Scavenging System →

O_2^- (Superoxide)

↓ SOD → superoxide dismutase

H_2O_2 (mild free Radical)

$H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$

$H_2O_2 \xrightarrow{\text{Glutathione peroxidase}} 2H_2O$; (Reduced) 2GSH → (oxidized) GSSH

$H_2O_2 + Cl^- \xrightarrow{\text{myeloperoxidase}} HClO$ → Bacteria destroyed

er's Signature ....

Respiratory Burst

A large amount of O_2 is consumed by macrophages during their bactericidal function
⇓
this phenomenon is K/as "Respiratory Burst".

Neutrophil

"OH˙ & HClO" Both causes the Respiratory Burst.

O_2
↓ NADPH oxidase
O_2^-
↙ SOD
$H_2O_2 \xrightarrow{MPO} HClO$ ⟶ Hypochlorous acid
⇓
Very dangerous

Fe^{2+} → $OH^{\bullet}$

Very dangerous, K/as "Transition Metal"
⇓
Cu^{2+}, Fe^{2+}

Fenton Rx^n (converts Ferrous to Feeric)

Don't occur in free, unbound form.

Biotransformation & Detoxification of Xenobiotics

Process by which a Substance is changed to other by a chemical reaction within body

addition of some functional group

Phase I → Results in detoxification
⇓ attraction of molecule to add a functional group.
Oxidation;
Reduction;
Hydroxylation;

Phase II
⇓
Conjugation Rx

Phase I
→ Hydrolysis
→ Dealkylation
→ epioxidation.

Phase II
↓
· Conjugation Rxn
· Easily excretable

· conjugation with →
conjugate c̄ bilirubin ← glucuronic acid
glycine
Acetylation
γ-glutamyl-cysteinylGlycine ← cysteine
Tripeptide Glutathione ← Glutathione - if whole glutathione involve; it lies in phase-III
(Glu-cy-Gly)
↓
conjugation occurs c̄ cysteine of glutathione
Methylation
Glutamine
Sulphate - donated by PAPS (Phospho adenosine phospho sulphate)

Phase III → Very Rare
↳ when conjugation is with whole glutathione

· Cyto. 450 ⇒ Microsomal enzyme located on Smooth ER.

Most of the drugs (xenobiotics) are metabolized by CYP-450 located on Smooth ER.

• Enzymes ↑ Rate of Rx^n by ↓ activation Energy — Free energy difference b/w substrate & transition state

• Most coenzymes are derivatives of Vit. B — Thiamine (B_1); Riboflavin (B_2); Niacin; Pyridoxine (B_6), Biotin, Folic acid, Cyanocobalamin, Pantothenic acid.

Co-enzyme → organic

ENZYMES

Co-factor → Inorganic

Selenium → Glutathione Peroxidase

Cu^{+2} → Cytochrome oxidase, Lysyl oxidase, ceruloplasmin; Cyto C; Tyrosinase, ALA synthetase; MAO; SOD; Phenoloxidase (convert Fe^{+2} into Fe^{+3})

Zn^{+2} → Carbonic anhydrase, Carboxypeptidase, RNA Polymerase, Superoxide dismutase, Alcoholic dehydrogenase, Lactate dehydrogenase, Glutamate dehydrogenase, Alkaline phosphatase

Fe^{+2}/Fe^{+3} → Xanthine oxidase

Mo → Xanthine oxidase, Sulphite oxidase

Mg^{+2} → Phosphatase, kinase, Glutathione Synthetase.

* Ribozymes ⇓ enzyme, which is not a protein. • RNA Molecule

→ Holoenzyme (complete-enzyme)

- Protein part (Apoenzyme)
- Non-protein part
  - Co-enzyme
  - Co-factors

• Classification → OTHLIL

EC (Enzyme code)

EC → I	Oxidoreductase	
EC → II	Transferase	
cleavage of Bond with help of H_2O (irreversible) eg → Max^m GIT enzymes (Urease, Pepsin, Trypsin, Peptidase; Lipase) ← EC → III	Hydrolase	Main class
EC → IV	Lyase	Sub class
EC → V	Isomerase	Sub-sub class
EC → VI	Ligase	Individual enzymes

EC number consists of four digits → EC (A.B.C.D)

Urease ⇓ cut other CN bond; Pepsin, Trypsin ⇓ cut Peptide bond; Lipase ⇓ cut ester

(I) OxidoReductase → Involved in transfer of Hydrogen (oxidation & Reduction)

eg → Dehydrogenase → acceptor of hydrogen is coenzyme (NAD^+, $NADP^+$)

Oxidase → O_2 molecule - acceptor of hydrogen

Peroxidase → H_2O_2 molecule - acceptor of hydrogen

Oxygenase → Incorporating oxygen atom in the substrate molecule without the help of H_2.

Dioxygenase ⇓ Tryptophan Pyrrolase

eg ⇒ Hydroxylase ⇓ is monooxygenase.

Other eg → Catalase, Reductase

→ It doesn't take ... molecule

Teacher's Signature ...

* Prosthetic group ⇒ co-enzyme covalently bound c̄ enzyme
↳ Metal constitute M/c type of Prosthetic group

Date
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(II) Transferase :- Transfer any group other than Hydrogen
eg → All Kinase (Hexokinase; Glucokinase)
Branching enzyme ; DHL transacetylase (PDH complex)

(III) Lyase :- cleaves bond without H_2O [Reversible / Irreversible
↳ Atom elimination & leaving double bond behind ($-\overset{|}{C}=\overset{|}{C}-$)
eg → Aldolase
Fumarase
Argino succinate lyases
Decarboxylase
Synthase (if rxn is Reversible)
↳ $AB \rightleftharpoons A+B$
Hydratase & Dehydratase NEET'16

(IV) Ligase :- Two molecules are united with the help of ATP
eg → Synthetase (PRPP Synthetase) ┌ Arginosuccinate synthase
Carboxylase (Pyruvate carboxylase)

(V) Isomerase :- Atom in a compound is Redistributed (Geometrical Reorganisation
eg → Mutase
epimerase
Aldo-keto isomerase

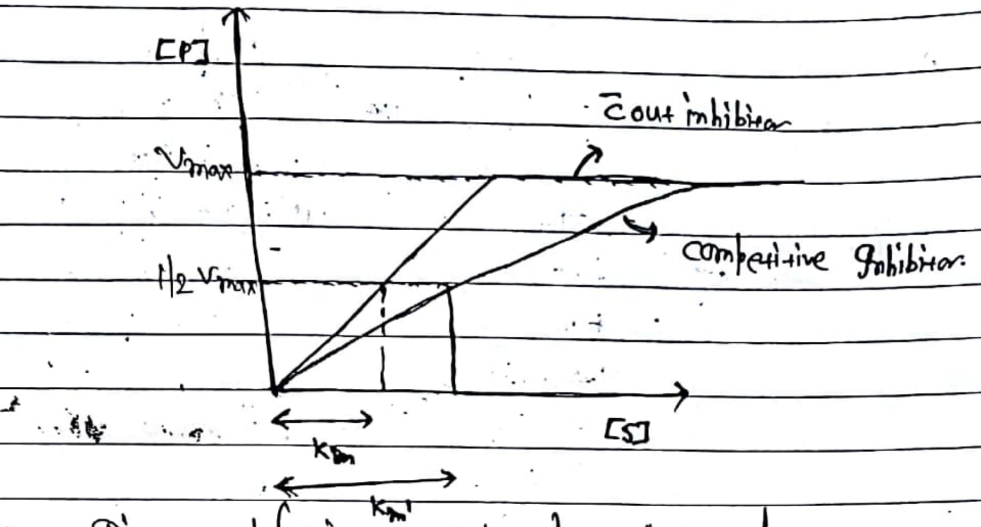
ENZYME INHIBITORS

1. Competitive :- They are drugs during Drick formation

Vmax →	Same
Km →	Incresed

eg → Malonate Inhibiting Succinate dehydrogenase
Sulpha drug as a PABA analogue
↓
Req. by bacteria for Folic acid synthesis
Statin → HMG CoA Reductase Inhibition
Methotrexate → DHF Reductase Inhibition.
Transaconitase → Inhibition of Aconitase
5-Flurouracil → Thymidylate synthase

The Substrate bind to Active sites by "Non covalent" Interactions like H-bond; Ionic (electrostatic) bond & Hydrophobic bond.

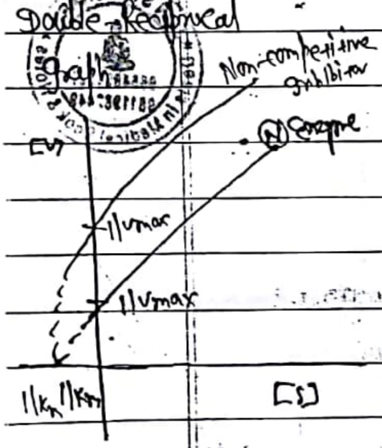


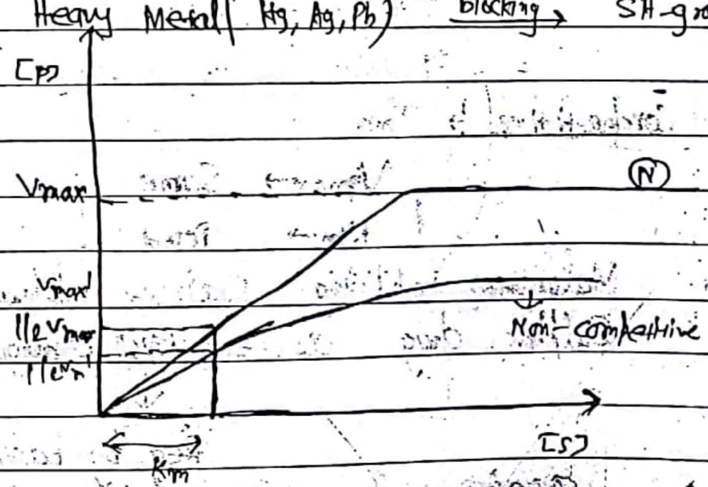
- Dicumarol (Vit. K Inhibitor) ⇒ Anticoagulant

2. Non competitive / Mixed type of Inhibitors (QQ) — They are Poisons Mainly as trick to Memorize

V_{max} →	↓se
K_m →	Same

eg → • Organophosphate Poisoning as AchEase Inhibitor
- Cyanide as Cytochrome Oxidase (cyto c) inhibitor
- Fluoride as Enolase Inhibitor
- Iodoacetate as Glyceraldehyde-3 PO4 inhibitor
- Heavy Metal (Hg, Ag, Pb) blocking → SH-group

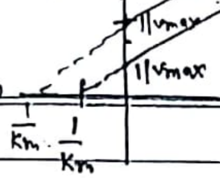


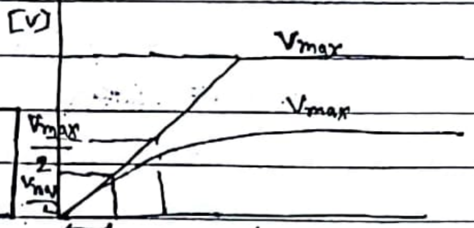


Teacher's Signature ....

Abzyme → Antibody with catalytic activity.

In Double Reciprocal graph





3. Uncompetitive

Vmax → ↓es
Km → ↓es

eg → Inhibition of Placental ALP by Phenylalanine

4. Suicidal inhibition :⇒ Enzyme own catalytic activity to convert a less potent Inhibitor → More potent inhibitor.

Xanthine

Xanthine oxidase ↓ Uric acid

⊖

Allopurinol ↓ Xanthine oxidase → Alloxanthine

K/as " Mechanism based inactivation ": Drug become more poisonous by using the mechanism of some enzyme.

5. Irreversible inhibitor :→

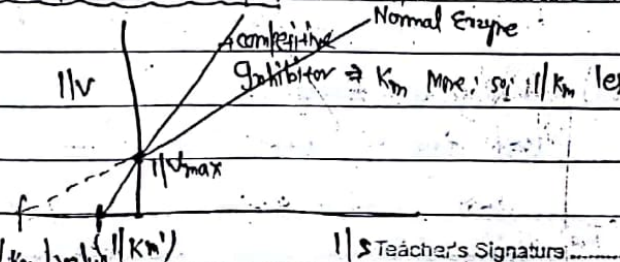
Km → No effect
Vmax → ↓es

eg → Cyanide inhibits cytochrome oxidase (Respiratory chain) Irreversibly & Non-competitively.

AIIMS * "cyclooxygenase" ⇒ K/as " Suicide enzyme" b/c it catalyzes its own destruction.

Lineweaver - Burke plot ⇒ K/as " Double Reciprocal plot" (b/w 1/v & 1/[S])

competitive inhibitor



NUTRITION

MICRO-NUTRIENTS

Macrominerals → It constitutes 60-80% of body's inorganic material.

↳ Ca^{+2}, PO_4^{-3}, Mg^{+2}, Na^{+}, K^{+}, Cl^{-} & SO_4^{-2}

↳ It requires in amount greater than 100 mg/day

Microminerals → It requires in amount less than 100 mg/day

Essential trace elements	Possibly essential trace elements	Non-Essential trace elements
eg → Fe, Cu, I, Mn, Zn, Mo, Co, F, Se, Cr	eg → Ni, V, Cd, Ba	eg → Al, Pb, Hg, B, Si, Bi

→ Zn: Mn: Cu: Se ⇒ Antioxident Property

MACRO-NUTRIENTS

→ Carbohydrate → 65-80% of total Energy
Fats → 10-30% of energy intake
Proteins → 7-15% of energy intake

→ Respiratory Quotient (RQ) → Ratio of volume of CO_2 produced by a volume of O_2 consumed.

$$\boxed{RQ = \frac{CO_2 \text{ Produced}}{O_2 \text{ Consumed}}}$$

(High/Low) Carbohydrate = 1
Mixed diet = 0.85
Protein = 0.8
(Fat) Lipid = 0.7

Teacher's Signature ....

→ SPECIFIC DYNAMIC ACTION (SDA) :→

It is the extra heat production by the body, over and above the calculated caloric value, when a given food is metabolized by the body.

Calculated Energy from food	=	Actual Energy	+ SDA

Calorific value →

Carbohydrate → 4Kcal/gm
Protein → 4Kcal/gm
Lipid → 9Kcal/gm

After consumption of these product we get some less energy than calorific value, b'coz some energy is invested in digestion assimilation (SDA)

SDA → Protein → Max^m
→ 30% of its calorific value

→ Carbohydrate → 5% of its calorific value

→ Fat → 15% of its calorific value

→ Mixed diet 10% of its calorific value

→ SDA is extra energy consumed in digestion, absorption & assimilation of food

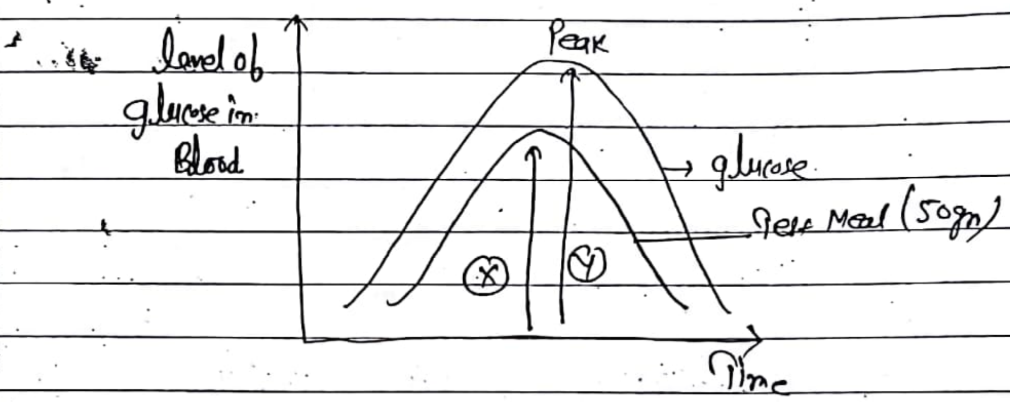
→ Thermogenic effect of food → ↑es in Metabolic Rate after ingestion of particular food. Result in an ↑es in the amount of heat generate by the body.

AIIMS - may 17 | Protein > Carbohydrate > Lipid | → order of thermogenic effect.

Glycemic index :-

give ~~50gm~~ glucose to person & assess blood glucose level over time.

Glucose tolerance curve :-



$$\boxed{\text{Glycemic index} = \frac{X}{Y}}$$

- Ratio of incremental area under the glucose tolerance curve after 50gm of Ingestion of test meal that of 50gm of glucose is "Glycemic index"

- Potato chips → 80-90
  Bread → 70-79 = White Rice
  Brown Rice → 60-69
  Banana → 60-69
  Legumes, Peanut → 25-40
  Milk, Icecream → 35-40

Less absorption hence less rise of blood glucose

Dietary fibre → The complex carbohydrates that are not digested by human enzymes

Insoluble fibre → prt. in vegetable & grain

↳ it absorbs H_2O & swell up

↳ it ↑es bulk of stool & ↓es transient time

eg → cellulose;
Hemicelluloses
Pectin;
Lignin;
grain;
gum;

Limiting AA :→ deficient AA

Pulses → Methionine [PM]
cereals → Lysine [CL]

Biological value :→ to assess the protein quality.

$$\boxed{BV = \frac{N_2 \text{ Retained}}{N_2 \text{ absorbed}}}$$

Net protein Utilization :→ Better Index to assess the protein quality ***

$$\boxed{NPU = \frac{N_2 \text{ Retained}}{N_2 \text{ Intake}} \times 100}$$

Teacher's Signature ....

VITAMINS

Fat Soluble → A, D, E, K

Vitamin A [Retinoids]

compounds having vit A like activity
- β-carotene
- Retinol
- Retinal
- Retinoic acid

Retinal oxidized → Retinoic acid

Retinal Reduced → Retinol

**** $\boxed{1\ \beta\text{-carotene} = 2\ \text{Retinol}}$

Sources → Liver oil, Buttermilk, cheese, egg yolk, Pumpkin, Tomato, carrot, Papaya, Mango, Sweet corn

RDA → 5000 IU/Day

- 1 Retinol equivalent = 1Mg of Retinol = 6 Mg β-carotene
- Antioxidant & anticancer
- Retinol absorbed in GIT & stored in liver in the form of Retinyl Palmitate
- In man liver is the only organ where β-carotene is converted to Vit A

(Stored in liver as Retinol ester)

A, E → Antioxidant

(90)

Role → ~~Growth, vision, differentiation, Reproduction.~~

- Wald's visual cycle → K/as "Rhodopsin cycle".

Rhodopsin (11 cis-Retinal-Opsin) → Light (photon) → Nerve impulse

Opsin

11-cis-Retinal ⇄ (Retinal Isomerase) All-trans Retinal

NADH+H⁺ / NAD⁺ — Alcohol dehydrogenase

NADH+H⁺ / NAD+ — Alcohol dehydrogenase (Liver)

11 cis-Retinal ⇄ Isomerase (Liver) All trans-Retinal

- Active form → 11 cis-retinal
  ↓
  When combined with opsin form Rhodopsin

- Inactive form → All trans Retinal

- Deficiency of vit. A → Night blindness (Nyctalopia)

dryness in conjunctiva & cornea ← Xerophthalmia

- White triangular plaques in certain area of conjunctiva ← Bitot's spot

Urinary calculi

Keratinisation of skin

destruction of cornea, causing total blindness ← Keratomalacia

Teacher's Signature ....

Date ______
Page 178

- Blindness

- Sterility in males → due to degeneration of germinal epithelium

- Hyperritaminosis A → Rupture of lysosomal membrane;

  - Pseudomotor cerebri

NEET'16 "Headache & Papilledema" - Bony Swelling
are found to be caused by increase in ICT.

Retinoic acid (action) → Gene expression;

Tissue differentiation

Glycoprotein Synthesis

Mucopolysaccharide Synthesis

Prevents keratinization

Prevents collagen breakdown

Vit A acts via → RAR (Retinoic acid Receptor) } Nuclear
RXR (Retinoid X Receptor) } Receptor

form dimer

QQ Actions at Nuclear Receptor → Vit A & Vit D

Teacher's Signature........

Vit. D

- Prohormone
- Synthesized in body
- Major storage form → 25, OH Cholecalciferol (Calcidiol) ↓ in liver
- Most potent form – 1, 25 Dihydroxy cholecalciferol (Calcitriol) ↓ Active form

Liver, Kidney

Biosynthesis of active form of vit. D →

7-Dehydrocholesterol (in Animals)

↓ Sunlight ⟶ Skin

Cholecalciferol (D3, Calciol)

↓ calciol 25-hydroxylase (liver)

25-OH cholecalciferol (calcidiol)

↓ calcidiol 1α-hydroxylase (kidney)

1,25-Dihydroxy cholecalciferol (calcitriol) ⟶ active form.

If Ca^{+2} ↑ ⟶ 24,25 di-OH cholecalciferol formed (inactive)

Date
Page 180

Best Source (NEET'16)

Sources → Fish liver oil, egg yolk, Margarine

ProvitD → 7 dehydrocholesterol, ergosterol
↓
(+) in skin → absorb UV → calcidiol

RDA → Adult → 200 IU/day

Pregnant, Lactation, children = 400 IU/day

Old age = 600 IU/day

Role of vit.D → • GIT absorption of Ca^{++} & PO_4^{-3} & send them for Bone mineralisation

• Renal absorption of Ca^{++} & PO_4^{-3}

• Excess dose of vit.D → toxic

Deficiency of vit.D → Rickets in children & Osteomalacia in adults

Rickets → Bow legs;
Plasma level of calcitriol
Alkaline phosphatase activity elevated;

* Excess dose for vit.A & vit.D both are toxic

Teacher's Signature ....

Vitamin E (Tocopherol)

- Anti-infertility & anti-oxident
- α-tocopherol → Most active form
  ↳ Most potent antioxident.

Sources → Cotton seed oil;
Corn oil;
Sunflower oil;
Wheat germ oil;
Margarine;
Soyabean;
Cabbage

RDA → Children → 10-15 IU/day (7mg/day)
Adults → 20-25 IU/day (10mg/day)

Role → Free Radical Scavenger
Reproduction in Rats

Therapeutically used in → Nocturnal Muscle cramp (NMC);
Intrahepatic cholestasis (IC);
Fibrocystic breast disease (FBD);
Atherosclerosis

Selenium → Vit E sparing effect (vit E like action)

Prevent Rancidity of fat

Teacher's Signature ....

compliments by liver → C_3, C_6, C_9

Date
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Vitamin K → ~~Function depend on calcium~~ QQ

three different form :-

K_1 → Phylloquinone (alfaalfa, Spinach, cauliflower, cabbage, Soyabean, tomato)

K_2 → Menaquinone (least potent) - by GIT bacteria

K_3 → Menadione (Most potent) - Synthetic Vit. K (Injection)

Vit. K crosses placenta & is available to fetus.

Role of vit. K → γ-carboxylation of glutamic acid residue to clotting factor 2, 7, 9, 10 * & calcium binding Protein (Osteocalcin, Matrix Gla Protein) & Protein C, S, Z

• oxidative Phosphorylation

RDA → 50 - 100 mg/day

Immediate inj. of vit K to New born

Deficiency → Hemorrhagic disease of Newborn.

Antagonist → Dicumarol, salicylates, heparin, bishydroxy-coumarin, warfarin

• Vit. K functions as a co-factor for the enzyme carboxylase QQ that catalyzes post-translational modification QQ of glutamate residues

Teacher's Signature ....

Vit A → Isotretinoic acid → Teratogenic ~~cleft lib~~
↓
~~Contraindicated in Pregnancy~~

Vitamin C (Ascorbic acid)
↓

- ~~Heat & alkali labile~~ Max^m in adrenal cortex
- ~~Versatile vitamin~~
- It is hexose derivative & closely Resembles mono-saccharide in structure

Source → Amla, Guava, Lime

RDA → ~~75mg~~/day

- Involved in hydroxylation of lysine & Proline (Post translational Modification)

Req for → Synthesis of epinephrine
Tyrosine Metabolism

Richest Source → Indian gooseberry (Amla)

Teacher's Signature ....

AI-B → 5 of "B" vitamins participate in the release of energy from carbohydrate, fat & proteins ⇒ B_1, B_2, B_3, B_5, B_7.

"Anti-Neuritic vitamin"

Vitamin B_1 (Thiamine)

→ also known as " Aneurine , Anti-beri-beri factor."

→ Found in "aleurone layer of cereals"

↓

Outer layer → Removed by Polishing; So, unpolished Rice is good Source

→ Made of Pyrimidine Ring & Thiazole Ring

→ Source → Cereals, Pulses, Liver, Pork, Meat, egg: Milk etc

→ TPP → Thiamine Pyrophosphate

- coenzyme of
  1. PDH complex;
  2. α-Ketoglutarate dehydrogenase complex;
  3. Branched chain α-Ketoglutarate dehydrogenase;
  4. Q Transketolase;
  5. Tryptophan Pyrrolase
  6. Pyruvate carboxylase

→ **"RBC transketolase" activity is used to assess Vit. B_1 in the body

↓ RBC transketolase activity

↓

Earliest Manifestation of Vit. B_1 deficiency

→ RDA = 1.5 mg/day

Deficiency → Beri-Beri :
Wernicke's Korsakoff's Psychosis :
Lactic Acidosis (Pyruvate ⨯→ Acetyl CoA
Pyruvate → LA)

Vitamin B_2 (Riboflavin)

- Yellow vitamin of Warburg.
- D-Ribitol attached to 6,7-dimethyl isoalloxazine by Nitrogen
  isoalloxazine → heterocyclic 3 Ring structure
- FMN, FAD are its two Coenzyme form.
- FMN → Cyto.c Reductase, L-amino acid oxidase
- FAD → Xanthine oxidase
  D-amino acid oxidase
  Aldehyde oxidase
  Succinate dehydrogenase
  Glycine oxidase
  Acyl CoA dehydrogenase

Riboflavin status
↓
AI-13
Glutathione Reductase

→ RDA → 1.5 mg/day

→ Deficiency → Glossitis, cheliosis, Angular stomatitis, circumcorneal vascularization, Proliferation of bulbar conjunctiva, Seborrheic dermatitis

→ Source → Liver, Yeast, egg, milk, fish, cereals

Teacher's Signature ....

Riboflavin → FAD | FMN
Niacin → NAD | NADP
→ oxid^n – Red^n Rx^n

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Synthesized c̄ in body from Tryptophan.

Vitamin B_3 (Niacin) → K/a "Endogenous vitamin"

Pellagra Preventing factor of Goldberger.

NAD, NMN & $NADP^+$ → Coenzyme form

→ NAD^+ →
- Isocitrate dehydrogenase
- alcohol dehydrogenase
- Lactate dehydrogenase
- Malate dehydrogenase
- Glyceraldehyde – 3 – P – dehydrogenase
- PDH complex
- α KG Dehydrogenase complex
- β-hydroxy acyl CoA DH

→ function as ADP – ribose donor for ADP ribosylation
↓
DNA Repair Mechanism

→ $NADP^+$ →
- G6PD
- Glutathione Reductase

→ Either NAD^+ or $NADP^+$ →
- Glutamate dehydrogenase
- Isocitrate dehydrogenase – cytosolic Need NADP; Mitochondrial NAD

→ Deficiency → Pellagra → Dementia, Diarrhoea, Dermatitis, Death.

→ RDA → 20 mg/day.

Teacher's Signature

NEET 16 * High Leucine cause Niacin deficiency.

* Pellagra is characterized by Hypersensitivity to Sunlight (Sun exposed area present c̄ erythema, ~~Desquamation & hyperkeratosis – hence presents as "Casal Necklace"~~).

Source → ~~Yeast~~, Rice, Liver, Peanut, Whole cereal, Legumes.

Pellagra Seen in:
- Hartnup's disease
- carcinoid
- Maize eaters

} Tryptophan deficiency

** $\boxed{\text{Tryptophan (60mg)} \xrightarrow{B_6} \text{Niacin } (B_3) \text{ (1mg)}}$

Vitamin B_5 (Pantothenic acid)

→ Act as cofactor after modification to Pantotheine

Pantothenate + cysteine → Pantotheine

→ K/as "Filtrate factor / chick anti dermatitis factor"

→ Consist of β-alanine & Pantoic acid

CoA → Active form [CoA-SH] Active moiety of CoA
↓ QQ
Thiol of Pantotheine

→ Richest Source → Royal jelly

→ Deficiency → Burning feet Syndrome

→ RDA → 10 mg / 2500 cal $\boxed{\text{CoA} \rightarrow \text{Acetylation}}$

→ deficiency is very Rare b/c it is present in almost all food items

Teacher's Signature

Vitamin B_6 (Pyridoxine)

→ Rat Anti dermatitis factor
→ Pyridoxine, Pyridoxal, Pyridoxamine (vitamers of B_6)
→ Source → Yeast, Rice Polishing, Seed, grains, egg yolk.
→ Richest Source → Royal jelly
→ Tryptophan Load test QQ
— High dose of Pyridoxine → Neurotoxicity
— B_6 Requirement ↑es with ↑ in intake of Protein.
→ Role of B_6 → · Transaldolase activity
· Transamination
· Decarboxylation of AA
↳ Glutamate → GABA
Histidine → Histamine
5-OH Tryptophan → Serotonin
Cysteine → Taurine
Serine → Ethanolamine

→ deficiency of Pyridoxine causes Niacin deficiency
→ Sulphur containing AA formation requires B_6 (PLP)
– cystathionine β-synthase
– cystathionase

→ ALA Synthase
AIIMS Nov'13 → Glycogen phosphorylase
Kynureninase

$\left.\begin{array}{l}\text{ALA Synthase}\\ \text{Kynureninase}\\ \text{Glycogen phosphorylase}\end{array}\right\}$ PLP co-enzyme

→ RDA → 1.5 mg/day

→ Deficiency → Epileptic convulsion in infant
Hypochromic Microcytic Anemia

→ Assessment of B_6 status → RBC transaminase activity
- Xanthurenic acid index

Vitamin B_7 (vit. H / Biotin / coenzyme R / Bios)

→ Anti egg white injury factor

→ Heterocyclic, Monocarboxylic Sulphur containing

→ Needed for *Carboxylation* Reaction :-

- Acetyl CoA Carboxylase
- Propionyl CoA Carboxylase
- Pyruvate Carboxylase
- β-Methyl Crotonyl CoA Carboxylase

→ Deficiency → Leiner's disease
→ RDA → 200-300 mg/day
→ Source → Gut Bacteria, Yeast, Peanut, Soyabean, Yolk

Uncooked egg white (Avidin) → ⊖ Biotin absorption

Date
Page 190

Vitamin B9 | Folic acid | Vit M | Fermentation Residue factor

→ Folic acid consists of three components

Pteridine Ring | PABA | glutamic acid

PABA ↓ p-amino benzoic acid

→ Imp. for "one-carbon Metabolism"

↓ use as carrier

Major form of Folic acid to transfer 1-C unit ↳ Methylene THF.

→ Active form → THF (FH_4)

AI-13 Blood form of Folic Acid → Methyl THF.*

→ RDA → 200 Mg/day
400 Mg/day (in Pregnancy)
300 Mg/day (in Lactation)

→ FIGLU excretion test / Histidine Load test

→ { Trimethoprim (component of drug septran or bactrim)
Pyrimethamine (Antimalarial drug)

→ Structurally Related to folic acid

Vitamin B12 (COBALAMIN)

- Red Vitamine
- Antipernicious anemia factor / Extrinsic factor of castle / Animal protein factor
- Corrin Ring with central cobalt atom.
- Forms → Cyanocobalamine
  Methyl cobalamine
  Hydroxy cobalamine
  Adenosyl cobalamine

→ Source → Food of animal origin are the only Source

→ Strict vegeterians → Vit B12 deficiency.

NEET'16
→ only water soluble vitamin stored in liver (2.5mg)
↓
Used for years; as requirement is very less

→ RDA → 2-3 Mg/day

→ Absorption → Intrinsic factor (Parietal cell of stomach)
+
B12 (extrinsic factor)
⇓
Ileum

Teacher's Signature ....

Action →

NEET'16 ① Synthesis of methionine from homocysteine :→

$$\text{Homocysteine} \xrightarrow[\text{Enzyme}]{B_{12} + FA} \text{Methionine}$$

Enzyme ↓ Homocysteine Methyltransferase

N^5-Methyl THF → THF

NEET'16 ② Isomerization of Methylmalonyl CoA to Succinyl CoA :→

$$\text{MMCoA} \xrightarrow[B_{12}]{\text{Isomerization}} \text{Succinyl CoA}$$

Deficiency :→ ① Pernicious Anemia;

NEET'16 ② Methyl malonyl aciduria;

③ SCAD (Short-chain Acyl CoA dehydrogenase deficiency)

NEET'16 ④ Folate trap (Most of FA is trapped as N_5 methyl THF)

$$\text{Methyl THF} \xrightarrow{B_{12}} \text{THF}$$

Q. Major circulating form of folic acid ⇒ Methyl THF

Q. Major form of FA to transfer 1C → Methylene THF

Teacher's Signature

- Chromium, Zinc → Insulin
  Cobalt → B_{12}
  Copper → Lysyl oxidase

- Vitamin involved in Energy production :→
  B_1, B_2, B_3, B_5, B_7

- Post- translational Modification :→

NEET '16
Vit. B_7 & K → Carboxylation
Vit. C → Hydroxylation
B_5 → Acetylation

CORT'S CYCLE → Seen b/w Exercising Muscle
Lactic Acid cycle & Liver

Anaerobic glycolysis in Muscle = Lactic Acid
Muscle

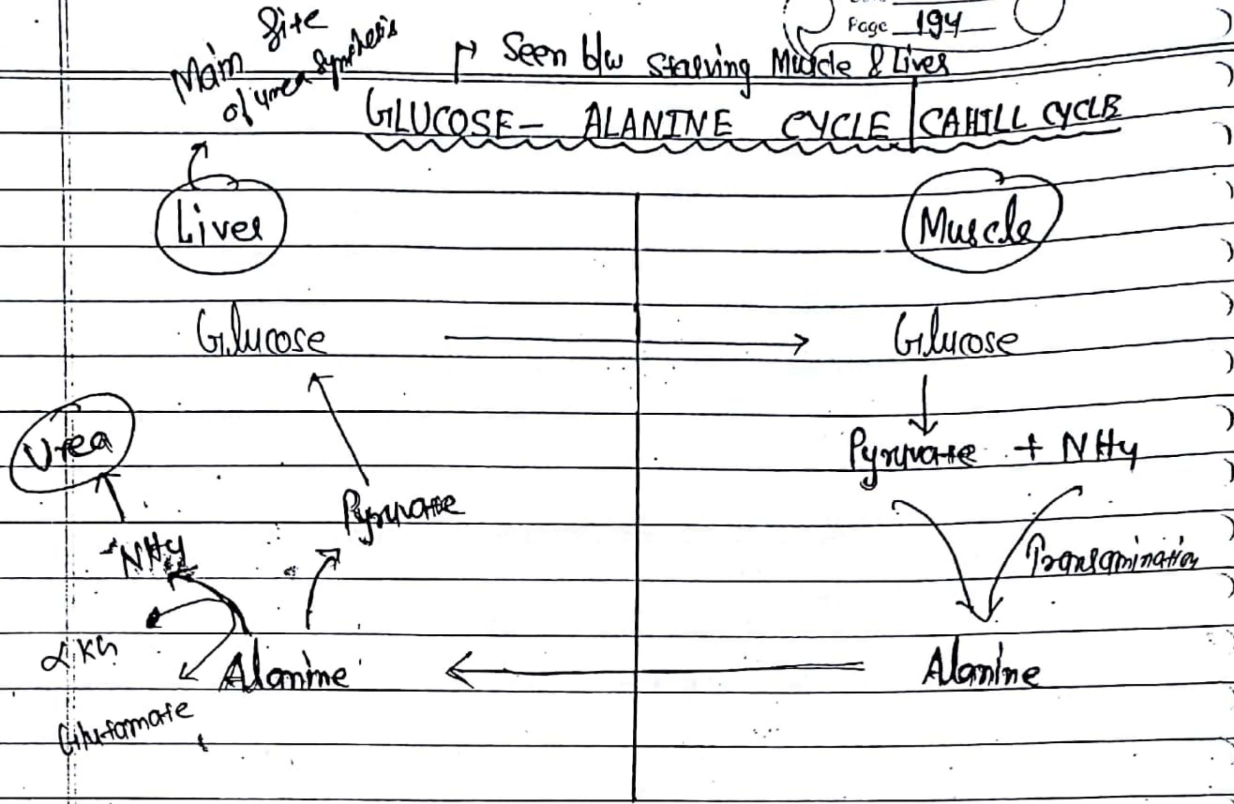
Glucose $\xleftarrow{\text{Gluconeogenesis}}$ Pyruvate ← Lactic acid
Liver

- Gluconeogenesis doesn't occur in Muscle

- Cycle involves → Synthesis of glucose in liver from the skeletal muscle lactate
  &
  the Reuse of glucose by the muscle for energy purpose

↑ Seen b/w starving Muscle & Liver

GLUCOSE- ALANINE CYCLE | CAHILL CYCLE



Other site of Urea Syn. → Brain, kidney

Alanine → Transport ammonia (excretion of NH_4^+)
Alanine → Gluconeogenesis (during fasting)
↳ Major AA released from muscle to liver during starvation

∴ Starvation → Serum alanine ↑

ONE LINEAR QUESTIONS

- Suicidal inhibitor → Allopurinol
  Suicidal enzyme → cyclo-oxygenase
- Protein Kinase phosphorylates → STT → Mnemonics
  Serine
  Threonine
  Tyrosine
- Iso-enzymes → Multimeric complex
  ↳ everything different → but Catalyse the Same rxn → Same Substrate
  ↳ Specific tissue distribution
- Iso-enzyme of Lactate dehydrogenase (LDH) :→
  $LDH_1 > LDH_2$ → MI
  LDH_4, LDH_5 → Liver, Muscle
  LDH_2 → N healthy individual
- LCAT (Lecithin- cholesterol acyltransferase) → Induced by Apo-AI
- Vitamin which act as Reducing agent → Vit-C
- 1st Response to Hypoglycemia → ↓ Insulin
- Allosteric Inhibition → Binding of inhibitor to other site & inhibition of Enzyme

Teacher's Signature ....

Rate limiting Enzyme :-

1. Bile Acid Synthesis → 7α hydroxylase
2. Catecholamine Synthesis → Tyrosine hydroxylase
3. Urea Synthesis → CPS-I
4. Fatty acid Synthesis → Acetyl CoA Carboxylase
5. AI-15 Cholesterol Synthesis → HMG CoA Reductase
6. AI-15 Ketone body Synthesis → HMG CoA Synthetase
7. Glycolysis → Phosphofructokinase

- SGPT / ALT → cytosomal enzyme
  → more specific for liver disease.

- SGOT / AST → Cytosol & Mitochondrial enzyme
  → more specific for heart disease.

- Serine Proteases → Endopeptidase
  ↓
  eg → Trypsin, Chymotrypsin, Elastase } Serine at active site

- Serine Residue → Catalytic activity
  - Hydrolysis peptide bond by involving Carbonyl group.

- Carboxyl protease → eg Pepsin
- Chymotrypsin cleaves carboxyl group of → Tryptophan, Tyrosine, Phenylalanine

AI→15 High energy compounds :→ ≥ 7 kcal/mol
↓
eg → PEP → 14.8 Kcal/mol
Carbamoyl phosphate → 12.3 Kcal/mol
cyclic AMP → 12.0 Kcal/mol
ATP → 7.3 Kcal/mol

ETC INHIBITOR

Complex I → C → Chlorpromazine / P → Piericidin A
A → Amytal (Barbiturate drug) or A → Amytal
R → Rotenone / R → Rotenone

Complex II → Malonate, Carboxin

Complex III → B → BAL (British Antilewisite)
A → Antimycin
P → Peforin

Complex IV → CO, CN, H_2S, Azide → CO → Reacts c̄ reduced form of cytochrome;
CN & Azide → c̄ oxidized form of cytochrome

Atractyloside – Inhibitor of Nucleotide transporter
↳ ⊖ oxidative phosphorylation

Teacher's Signature ....

- Cytosolic Pathways → Glycolysis (EMP);
  → HMP Shunt
  → Fatty acid Synthesis;
  → Glycogenesis;
  → Glycogenolysis;
  → Bile acid / Salt Synthesis
  → Cholesterol Synthesis

- Mitochondria → TCA cycle
  ETC
  FA oxidation
  Ketogenesis

- Both cytosol & Mitochondria → Gluconeogenesis
  Urea cycle

- Peroxisomes – Oxidation of Very long chain Fatty acid (VLCFA)

- Smooth Endoplasmic Reticulum → Triglyceride Synthesis
  Steroid synthesis
  Cholesterol synthesis

* Human cells Lacks enzyme beyond Δ_9 desaturase; so beyond Δ_9 EFA.
* Linoleic acid makes Arachidonic Acid ; so it is semi-essential / Linoleic acid gone after making γ- Linolenic acid.

Lipids – 3 types

Simple	Compound (complex)	Derived lipid
- Glycerolipid	- Phospholipid	- Fat soluble vitamin
- Sphingolipid	- Glycolipid	- Steroid hormones
	- Lipoprotein	- Ketone bodies

→ Monoenoic acid → Oleic acid (18C)
↳ Unsaturated FA having only one double bond
↳ Δ_9 position

→ Fatty acid with max^m C → Cervonic acid > Arachidonic acid
(22C) (20C)

They have Anti-inflammatory effects

Docosa Hexanoic acid

18:3 (Δ_9, Δ_{12}, Δ_{15})

→ ω_3 FA	ω_6 FA	ω_9 FA
Cervonic Acid (DHA)	Linoleic acid (18:2 (Δ_9, Δ_{12}))	(C18) Oleic acid (18:1 (Δ_9))
α-Linolenic acid	γ-Linolenic acid	NEET'16 Elaidic acid (trans-fatty Acid)
Timnodonic acid (Eicosa Pentanoic Acid)	Arachidonic acid ↳ Semi-essential FA	(18:1 (Δ_9))

→ Human can't Synthesize → Linoleic acid
Linolenic acid
so, essential in diet.

→ Most essential FA → Linoleic acid

→ FA present exclusively in Breast milk →
Docosahexanoic acid ⇒ also in Fish oil.
↓
Req. for development of CNS & visual abilities

→ Trans fatty acids → It ↑es risk of cardiovascular disease.
→ formed by hydrolysis of vegetable oils.
In fried food → TFA ↑
Oil Refining → TFA ↑
→ It increase LDL & decrease HDL

Rancidity → Deterioration of food

→ Cardioprotective FA → Unsaturated FA

ω_3 FA ← (PUFA) → MUFA (oleic acid)

↓

Max^m

→ Saponification → Hydrolysis of fat by alkali

→ Auto-oxidation (Peroxidation) → Seen with PUFA

↓ ↓

Responsible for "Rancidity" of food. (eg. Arachidonic acid)

→ Medium chain FA / Short chain FA →

- don't required Pancreatic lipase, Bile salt for digestion;
- Directly absorbed into portal circulation;
- oxidized by Peripheral cells;
- Not used for storage.
- stops chyluria

→ Large chain FA →

- Required enzyme & Bile salt for digestion.
- taken by Lymphatics
- Storage form of lipid (Triacylglycerol)

PHOSPHOLIPID

	Glycerophospholipid	Sphingophospholipid
alcohol →	Glycerol	Sphingol
eg →	Phosphatidylcholine (Lecithin)	eg → Sphingomyelin
	Plasmalogen	
	Cardiolipin	

→ Sphingomyelin → FA + Sphingosine + Phosphoric acid + choline

$\underbrace{\text{FA + Sphingosine}}_{\text{Ceramide}}$

Teacher's Signature

→ Glycosphingolipid = Ceramide (FA + Sphingol)
↓ +
Cerebroside glucose

→ Cardiolipin → Major phospholipid of inner Mitochondrial membrane
↳ It is Antigenic

→ Ganglioside → Most complex form of Glycosphingolipid
↳ Ceramide + carbohydrate + NANA

Sphingol + Long chain FA — oligosaccharide chain (glu + gal) — Sialic acid

→ Sphingolipids are chiefly accumulated in
↳ CNS (Brain + Nerve)

→ Phospholipid
- with glycerol as alcohol group
  ↳ Lecithin
  cardiolipin etc
- With Sphingosine as alcohol group
  ↳ Sphingomyelin
  Cerebroside
  ganglioside
  globoside
  Ceramide

→ Lecithin = Phosphatidyl choline
↓ Hydrolysis
Glycerol 3P + choline

Teacher's Signature ....

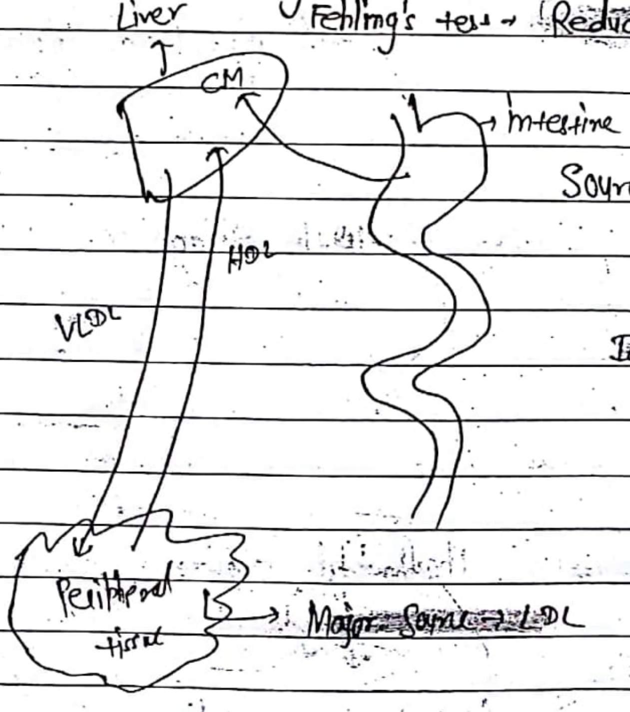
→ Insulin inhibits Ketogenesis by →

Inhibiting β-oxidation
Inhibiting Lipolysis
↑ esterification of Fatty acids
Directing Acetyl CO-A to TCA cycle

→ Ketone body → $\bar{c}$ glycosuria → DM
without glycosuria → Starvation

→ Ketoacidosis → presenting Sign & symptom → Dehydration
Altered Sensorium

→ Tests →
Benedict's test → Reducing Sugar in Urine
Rothera → Ketone bodies
Fouchet's → Bile Pigments
Hay Sulphur → Bile salt
Fehling's test → Reducing Sugar in Urine



Source → CM → Intestine
VLDL → Liver
CM Remnant → CM
IDL, LDL → VLDL

Major Source → LDL

Teacher's Signature ____

→ Protein absorb UV due to → Aromatic AA
↳ k/a " Photochromosity ".

→ AA with double chiral carbon → Isoleucine (IT)
Threonine

→ AA with No chiral carbon, No Anomeric carbon
→ Glycine

→ AA c̄ pKa ≈ pH ⇒ Histidine
↳ Most stable AA at Physiological pH.

→ Keratin → Fibrous Protein

→ Transport protein → Albumin, globulin
↓
GLOBULAR PROTEINS

→ SDS PAGE → For M_w determination
PAGE → dependent on Molecular charge & molecular size

→ DEAE cellulose chromatography → ⊕ve
↳ ⊕ve charge AA move fastest on this
CM cellulose chromatography → ⊖ve
↳ ⊖ve charge AA move faster on this

→ Methods to determine Protein structure →
- X-ray crystallography / diffraction (Study of chola)
- UV light / IR spectroscopy
- NMR spectroscopy
- Mass spectroscopy (peptides are studied by Projection of "hydrogen ion H^*")

→ Affinity electrophoresis → for protein-protein interaction

→ α-Amino acid $\xrightarrow[\text{PLP}]{\text{Transamination}}$ α-Keto acid
↓ ↓
DONOR ACCEPTOR
↳ Transfer of amino group

→ "PLP" (Pyridoxal phosphate) → Co-enzyme for Transamination

→ Removal of Amino group → Deamination

→ Glutamine → in Blood work as NH_3 transporter

→ Carrier of Ammonia (Nitrogen) from muscle to liver
↳ Alanine

→ Ammonia is detoxified in brain ⇒ by α KG to glutamine

Detoxification →

$$\alpha KG \rightleftharpoons Glutamate \xrightarrow{NH_4^+} Glutamine$$

In hyperammonaemia ⇒ More glutamate is required
↓
More α-KG consumed
↓
TCA cycle is inhibited

→ Glutamine → Major Source of NH_3 in Kidney.

→ $$Glutamine \xrightarrow{Glutaminase} Glutamate + NH_4^+$$

Source of NH_3 in urine → Glutaminase (Glutamine)

→ Oxidative deamination → Occurs in Mitochondria of hepatocytes & Kidney.
↓
Catalysed by → Glutamate dehydrogenase
↓
Cofactor → NAD

Deamination of glutamine occur in liver

Teacher's Signature :

Urea cycle – occurs in liver

- Starts in mitochondria, ends in cytosol
- Urea formed in cytosol.
- Source of Nitrogen – Aspartate & Ammonia

N-acetyl Glutamate

↓ (+)

$CO_2 + NH_3$

–2ATP ↓ carbamoyl phosphate synthase – I (CPS – I)

↓ (–)

Carbamoyl phosphate

Hyperammonemia type I

Ornithine $\xrightarrow{\text{ornithine Transcarbamoylase}}$ Citrulline (Mito)

(Cyto)

Citrulline

ATP, Aspartate

AMP + PPi ← Arginosuccinate Synthase

↕ (–)

Citrullinemia

Arginosuccinate

Arginosuccinase

Fumerate → used in TCA

Arginine

Arginase — Hydrolytic cleavage

Urea ← Ornithine

- Total 4 ATP are used
- Urea formed by hydrolysis of Arginine

→ Ornithine transcarbamoylase deficiency → ↑ Carbamoyl phosphate

↓

Orotic Acidemia ← Pyrimidine ↑ ← CPS II (+)

Teacher's Signature ....

VVI
AI-2K6

AA which can be converted to Succinyl CoA
V → Valine
I → Isoleucine
M → Methionine

→ Sulphur containing AA → cysteine, Methionine, homocysteine

Homocysteine $\xrightarrow[B_{12}]{PLP(B_6)}$ Methionine

N^5-Methyl THF → THF

∴ Homocystinuria → Abnormal Metabolism of Methionine

Starvation

→ At the time of starvation, Body uses → Ketone bodies

• Ketone body not used by →
 RBCs → No Mitochondria
 Liver → Lack CoA transferase required for activation of ketone bodies

• In Starvation → Glucose ↓
but still brain utilise glucose → due to ↓ K_m of Hexokinase
Liver doesn't utilise glucose → due to ↑ K_m of glucokinase

→ In Seriously ill patient → addition of AA in diet results in the N_2 Balance due to ↑es secretion of insulin ★
⇓
Glucose, Mannose, AA, β Keto acid ⇒ ↑ insulin secretion
⇓
(Insulin has anabolic effect) Protein Synthesis

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AIIMS 2014

In traumatic brain injury

⇓

Pyruvate DH activity ↓

& Pyruvate carboxylase activity ↑ for gluconeogen

Lactic acid ↑ in Brain & CSF

⇓

↓ uptake from circulation.

Earlier the fall in CSF lactate

⇓

Better Prognosis

Teacher's Signature

ONE-LINER QUESTIONS

- ** NEET** Aldehyde dehydrogenase requires "NAD" as "Co-enzyme"
- ** SNB** Enzyme Transketolase Requires "Thiamine Pyrophosphate (TPP)" as co-enzyme.
- ** AI 13** Vit. B_2 (Riboflavin) & Niacin; both vitamins are involved in "oxidation & Reduction process".

*

Co-enzyme
Works as

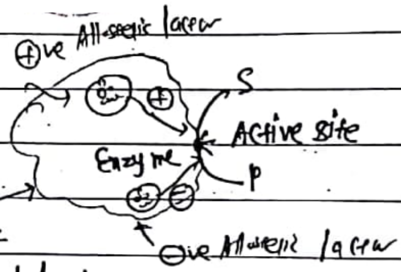
Co-Substrates (Second Substrate)	Prosthetic group
acts as either a donor or acceptor in a group transfer Reactions	It bounds permanently to the active site of its enzyme

⇓

Co-enzyme are associated c̄ active site of enzyme only "transiently"

⇓

M/c Mechanism by which co-enzymes act.



**

Allosteric Modulation

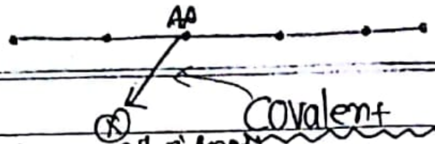
Allosteric enzymes → It posses a site, in addition to Substrate binding (catalytic) site, K/as "Allosteric site".

→ if allosteric Regulator

prevent the conformational change required for binding of Substrate.	facilitate the conformational change of catalytic site required for Substrate binding
⇓	⇓
Allosteric inhibitor	Allosteric activators
eg → Citrate is an allosteric inhibitor of Phosphofructokinase-I	eg → Fructose-2,6 bisphosphate is an allosteric activator of Phosphofructokinase-I

Teacher's Signature

* Fine control of enzymatic activity is possible c̄ Allosteric Modification Not in covalent Modification.



Covalent Modification (All or None phenomenon)

(Po4, So4; Ribose; Acetyl; Methyl; Ubiquitin)

→ M/c covalent Modification → Addition or Removal of a phosphate group.

→ Phosphorylation (Addition of a phosphate group) $\xrightarrow[\text{by}]{\text{catalyzed}}$ Protein kinase

Dephosphorylation (Removal of a phosphate group) $\xrightarrow[\text{by}]{\text{catalyzed}}$ Protein phosphatase

Enzyme	Active	Inactive
→ Glycogen synthase	Dephosphorylated	Phosphorylated
→ Pyruvate dehydrogenase	Dephosphorylated	Phosphorylated
→ HMG CoA Reductase	Dephosphorylated	Phosphorylated
→ Pyruvate Kinase	Dephosphorylated	Phosphorylated
→ Citrate lyase	Phosphorylated	Dephosphorylated
→ HMG CoA Reductase kinase	Phosphorylated	Dephosphorylated.
→ Glycogen phosphorylase	Phosphorylated	Dephosphorylated

N.B. Protein kinase mostly add phosphoryl group to serine, threonine, or Tyrosine Residues.

enzyme efficiency & specificity ⇒ Measured by

$\boxed{\text{Ratio of } K_{cat}/K_m}$

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Measurement of enzyme activity

① Unit of enzyme activity :→

One Unit of enzyme activity ⇒ defined as amount causing transformation of 1 μmole of substrate/min at 25°C:

② Specific activity → No. of enzyme Units per miligram of protein.

③ Turn over Number → No. of Substrate molecules transformed per unit time by a single enzyme molecule (or by a single catalytic site). denoted by "K_{cat}" (Measure catalytic activity)

Turn over No. → Catalase > carbonic Anhydrase > Acetylcholinesterase > Amylase > LDH > Trypsin > Chymotrypsin > DNA Polymerase > Lysozyme

Catalase → Highest turn over (so; fastest active enzyme).
Lysozyme → Lowest turn over (so; slowest acting enzyme)

ISOENZYMES (ISOZYMES)

→ They are physically distinct forms of the same enzyme.

→ They catalyze the same chemical reaction but differ from each other structurally, electrophoretically & immunologically

→ It possess quaternary structure and are made up of two or three different subunit (Multimeric)

Teacher's Signature ....

Physical differences ⇒ • Substrate affinity
• electrophoretic Mobility
• Immunological Properties.
• Km value also different
• Distribution → organ (ALP) → Bile canaliculus, Placenta, Liver, GIT, WBC
→ Compartmentalization
• Structure
Subunit association differs ← (Hybrid isoenzyme) eg = LDH, CPK

• It acts on Same Substrate, but with different Km & Vmax values i.e. isozymes have different kinetics.

• Tissue distribution of isoenzymes is quite specific. Q

1) Lactate Dehydrogenase (LDH) → LDH is tetramer with two types of polypeptide units: H (for heart) & M (for Muscle)

It has five isoenzyme → LDH1 (HHHH)

LDH2 (HHHM) → ↑es in Megaloblastic Anemia. Q

LDH3 (HHMM)

LDH4 (HMMM)

LDH5 (MMMM) → Predominant found in Skeletal Muscle
⇓
Predominant isoenzyme in Liver Q

• LDH-1 & LDH-2
⇓
Predominant isoenzymes in Myocardium; therefore they raised in MI (LDH1 > LDH2).
⇓
LDH1 is more specific for Myocardium than LDH2.

LDH2 > LD1 (Normal plasma)
⇓
Flipped pattern of LDH

→ Normal LDH Pattern on electrophoresis ⇒ LDH2 > LDH1 > LDH3 > LDH4 > LDH5 Q

2) Creatine Kinase (CK) → Dimer Made up of two type of Subunits (B and M).

It has three isoenzyme →
CK1 (BB) → Major isoenzyme in brain
CK2 (MB) → Major isoenzyme in Myocardium
CK3 (MM) → Major isoenzyme in skeletal Muscle

Total CPK is None of diagnostic value
do M, B separately

Teacher's Signature ....

True Isoenzyme : 1. True isoenzymes : ⇒ MDH → Cytoplasmic
→ Mitochondrial
(Different genes)
Amylase → salivary
→ Pancreatic
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2. Isoform isoenzymes :⇒ Post translational Modification
↳ ALP → sialic Acid

37) Acid phosphatase → it hydrolyzes phosphoric acid esters at pH 5-6 Q.

- Found in different isoforms in prostate, spleen, liver, erythrocytes, platelets & bones.

- Prostatic isoform → Inhibited by Tartarate Q

erythrocytic isoform → Inhibited by formaldehyde & cupric ions Q

PLASMA ENZYMES

→ Blood plasma contains enzymes, which are classified into functional & Non-functional plasma enzymes :

	Functional (plasma-derived)	Non-functional (cell-derived)
Concn in plasma →	Higher in comparision to tissue	Very low in comparison to tissue
Funcn →	Known functions	No known functions
Site of Synthesis →	Liver	Liver, heart, brain, skeletal muscle
clinical importance →	No	Diagnosis & Prognosis of disease
Examples →	Lipoprotein lipase Q, clotting factors Q, Pseudocholinesterase	ALT (SGPT), AST (SGOT), LDH, alkaline phosphatase, Acid phosphatase, gamma glutamyl transpeptidase, lipase

** Lipase is Non-functional enzyme but

Teacher's Signature

TRANSAMINASES

- It catalyze reversible transfer of an Amino group from an α-amino acids to an α-Keto acids.

(A) Aspartate transaminase (AST or aspartate Aminotransferase) :-

- K/as " Serum glutamic oxaloacetic transaminase (SGOT)
- Concⁿ of this enzyme is very high in Myocardium[Q] & in liver cells.
- It is found both in → Cytoplasm (AST or SGOT1) and Mitochondria (AST or SGOT2)
  - Cytoplasm ⇓ derives from heart & RBCs
  - Mitochondria ⇓ present predominantly in Liver.

(B) Alanine transaminase (ALT or Alanine aminotransferase) :-

- K/as " Serum glutamic Pyruvic transaminase (SGPT)".
- Mainly found in Liver[Q]. It is entirely cytoplasmic[Q]
- Both ALT & AST raised in liver disease; but ALT (SGPT) is always rise more than AST (SGOT).
- In Normal healthy person " AST is slightly more than ALT

Teacher's Signatu

ALKALINE PHOSPHATASE

- It is an ectoenzyme i.e localized to plasma membrane.
- It is found in liver, bone, kidney, intestinal Mucosa & Placenta.
- Raised ALP ⇒ useful in diagnosis of "Bone & Liver" Pathology.
- isoenzymes of ALP ⇒ (1) α-1 ALP → Produced in the "epithelial cells of biliary canaliculi".

(2) α-2 ALP → Produced by "hepatic cells".
(Heat labile)

(3) α-2 ALP → derived from "placenta".
(Heat stable)

(4) Pre-β ALP → It is of "Bone origin".

(5) Gamma ALP → Originates from "Intestinal cells".

(6) Leukocyte alkaline phosphatase (LAP) :- originates from "Leucocytes".

N.B. "Regan enzyme" → Isoenzyme of "Alkaline phosphatase".

PROTEASES

→ It catalyze the clevage of peptide bonds in proteins & peptide molecules with the participation of water as co-Reactant.

Classified on the basis of functionally essential critical groups or Residues at their active sites :-

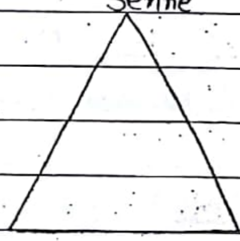
i) Serine proteases → posses "Serine Residue" at the active site.

↳ eg → Trypsin[Q], Chymo-trypsin[Q], elastase[Q], Thrombin[Q]

- They are inhibited by disopropylphosphofluridate which binds covalently to Serine Residue

- Catalytic Triad →

Active site of Serine protease contains these three Amino acids.



- Serine protease ⟶ Highly Reactive ; can damage itself

⇓

So, Secreted in zymogen (inactive) form

⇓

activated in the intestine by proteolytic activation

→ can't activated by autolytic (auto catalytic) activation ; otherwise they damage the cells

Serine Protease hydrolyze peptide bonds in 2 steps → (A) the highly Reactive serine 195 hydroxyl group attacks the carbonyl group of the substrate to form Acyl-enzyme intermediate

(B) Acyl-enzyme intermediate is hydrolyzed & Released the carboxylic acid component of substrate.

PG I M 2011

Chymotrypsin hydrolyses peptide bonds which are connected with carbonyl group of tryptophan, Tyrosine & phenylalanine.

Trypsine catalyses hydrolysis of Lysine & Arginine esters.

ii) Thiol or cysteine Protease :-

Active site cysteine Residue whose side chain -SH must Remain free for their activity

eg → papain, Caspases.

Inhibited by "iodoacetamide"

iii) Aspartate or carboxyl or Acid proteases :-

active site a critical carboxyl group from Aspartic acid & have acidic optimum pH.

eg → Pepsin, Lysosomal cathepsin & Proteases produced by HIV.

iv) Metallo proteases → Requires at the active site a tightly bound metal for their activity

eg → Carboxypeptidase A & B requires Zn^{2+}

Teacher's Signature ....

- NAD⁺ - Linked dehydrogenases → Pyruvate dehydrogenase;
  Isocitrate dehydrogenase; Q
  Malate dehydrogenase; Q
  α-Ketoglutarate dehydrogenase;
  Glutamate dehydrogenase;
  Glyceraldehyde-3 P dehydrogenase; Q
  Lactate dehydrogenase; Q
  β-hydroxyl acylCoA dehydrogenase; Q
  glycerol-3P-dehydrogenase (Cytoplasmic) Q

- NADP⁺ - Linked dehydrogenases → Glucose-6-P dehydrogenase;
  Gluconate dehydrogenase;
  3-ketoacyl Reductase;

- FAD - Linked dehydrogenases → Succinate dehydrogenase,
  glycerol-3P-dehydrogenase (Mitochondria) Q

→ High Energy compounds

(A) Phosphate compounds → Nucleotides (ATP, GTP, UTP, UDP-glucose);
Creatinine phosphates;
Carbamoyl phosphates;
Arginine phosphates;
Phospho enol Pyruvate;
Inorganic Pyrophosphate;
1,3-bisphosphoglycerate

(B) Sulfur compounds → CoA derivatives (Acetyl CoA, Succinyl CoA, HMG CoA,
S-adenosyl Methionine (SAM);
Adenosine phosphosulfate

GLUT → glucose transporter
SGLT → Sodium glucose ~~transporter~~

GLUCOSE TRANSPORT

There are two kinds of transport mechanisms :→

Facilitated transport (Bidirectional) | Secondary active transport (Unidirectional)

$GLUT_1$ → Brain, kidney, Placenta, erythrocytes NEET'11

$GLUT_2$ → Liver, Small Intestine, kidney, Pancreatic β cell

$GLUT_3$ → Brain, kidney, Placenta, Neuron NEET'16

V.Q.
$GLUT_4$ → Heart Q, Skeletal muscle Q, Adipose tissue Q

$GLUT_5$ → Small Intestine

$GLUT_8$ → Blastocyst, Testis; Brain (Transports glucose to Mature spermatozoa)

$GLUT_9$ → Liver; Kidney (Urate transporter)

$SGLT_1$ → Small Intestine & Kidney

$SGLT_2$ → Renal Tubules

$GLUT_4$ → Insulin - Stimulated glucose uptake

$GLUT_5$ → Absorption of fructose Q AI-14

$SGLT_1$ → Active uptake of glucose against a concⁿ gradient

ALCOHOL METABOLISM

→ Ethyl alcohol ⇒ absorbed from GIT & degraded by oxidation (oxidative process)

→ "liver" → Major site for ethanol oxidation

MEOS → Microsomal ethanol oxidizing system.

Cyto. P450

O_2

MEOS*

$NADPH + H^{+}$ → $NADP^{+}$

Ethanol $\xrightarrow{\text{Alcohol dehydrogenase}}$ Acetaldehyde $\xrightarrow{\text{Aldehyde Dehydrogenase}}$ Acetate

NAD^{+} → $NADH + H^{+}$

Catalase

⇒ ≃ 80% of acetate "leaves the Liver" & undergoes further metabolism in extrahepatic tissues like heart & skeletal Muscle.

⇒ ≃ 20% acetate is utilized in liver in lipogenesis.

→ Excess alcohol intake causes → ① Hypoglycemia**

⇓

Excess alcohol intake leads to excessive production of "NADH"

⇓

↑ed NADH favours Reduction of Pyruvate to Lactate & oxaloacetate to Malate

⇓

Result in ↓ed gluconeogenesis

Teacher's Signature ....

(2) Inhibition of β-oxidation of FA & TCA:

(3) ↑es lipogenesis, TG's synthesis & cholesterol synthesis from Acetyl Co-A

(4). Accumulations of lipids causing fatty liver, fatty myocardium & fatty kidney.

(5) Lactic Acidosis & Hyperuricemia.

Synthesis of creatine & creatinine

- Creatine and creatinine are not Amino Acids; but they are specialized products of AA
- Synthesized from → Glycine; Arginine; Methionine
- Synthesis takes place in kidney; Start with formation of "guanidoacetate from glycine & arginine in kidney"; Further rxn takes place in Liver and Muscle.

Arginine + glycine $\xrightarrow{\text{in Kidney}}$ Guanidoacetate (Glycocyamine)

Guanidoacetate $\xrightarrow[\text{SAM} \rightarrow \text{SAH}]{\text{in Liver}}$ Creatine

Creatine → creatine phosphate $\xrightarrow{\text{Muscle}}$ Creatinine

- Immediate precursor of creatine ⇒ Guanidinoacetate QQ
- Creatinine → Breakdown Product of creatine phosphate in Muscle QQ

⇓

- Quick Source of energy in Muscle:
- as a reservoir of high-energy phosphate groups.

- creatine phosphte $\xrightarrow{\text{ADP} \rightarrow \text{ATP}}$ creatine

⇓

- Helps in generation of ATP in exercising Muscles by Substrate level phosphorylation

GLUTATHIONE

- Tripeptide made up of "glutamate, cysteine & glycine" (γ-glutamyl – cysteinyl – glycine).

- Present in all mammalian cells except → Neurons;

- "Sulphydryl (–SH) group of cysteine residue"
  ⇓
  Reactive portion of glutathione, which can undergo oxidation & Reduction.

- Functions → • helps in keeping some enzyme in active state; by preventing the oxidation of sulphydryl group of enzyme
  - Scavenges free Radicals & Superoxide Anion.
  - Detoxification of xenobiotics by their conjugation (conjugation Rxn),
  - Prevent formation of Methemoglobin.
  - involved in the transport of AA across the cell membrane of Kidney & Intestine

Teacher's Signature ....

CM cellulose chromatography → CM (carboxy Methylcellulose) is ⊖vely charged; so; Negatively charged particles will move fastest b/c of Repulsion from ⊖ve charge CM.

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SEPARATION AND PURIFICATION OF PROTEINS

Methods → chromatography; centrifugation (Ultracentrifugation), electrophoresis, Ultrafiltration & Salt extraction.

Property of Protein used	Method
→ Protein Solubility	→ Salting out or salt extraction (precipitation of protein by ammonium sulfate) QQ
→ Molecular Size QQQ	→ Gel filtration chromatography Q (Agrose (sepharose); dextran (sephadex) are used.) → Ultracentrifugation → SDS-PAGE (Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis); → Dialysis
→ Molecular charge (Ionic charge)	→ Ion exchange chromatography QQ → High performance liquid chromatography (HPLC) → Electrophoresis. → Isoelectric focusing
→ Molecular Charge & Molecular weight	→ PAGE (Polyacrylamide gel electrophoresis)
→ Affinity Binding	→ Affinity chromatography
→ Hydrophobicity	→ Hydrophobic interaction chromatography.

"Diethylaminoethyl"

DEAE cellulose chromatography → DEAE groups have ⊕ve charges.

↳ ⊖ve charge proteins associate with ⊕ve charge DEAE groups & Replaced Cl⁻ ions.

CM cellulose chromatography → CM groups have ⊖ve charges; therefore they separate ⊕ve charged proteins i.e cation exchange.

Teacher's Signature

METHOD OF PROTEIN PRECIPITATATION

- Polarity (precipitation with ethanol or Acetone or Trichloroacetic Acid) QQ
- pH (Iso-electric) precipitation;
- Salt condn (Salting out c̄ ammonium sulfate);
- By Heavy metals (Pb, Hg, Cd).
- Heat induced precipitation (For large scale plasmid purification)

TRANSAMINATION

occur via "Ping-Pong Mechanism".

- Involves the Reversible transfer of α-amino group of α-amino acid to an α-keto acid to form a New Amino acid and a new keto acid.

Transaminase (Amino transferase) catalyze the Reaction;
↳ Requires "Pyridoxal phosphate (vit. B_6) as coenzyme".

Alanine Transaminase (ALT) / glutamate Pyruvate transaminase (GPT)
↳

$$\text{L-Alanine} + \alpha\text{-Ketoglutarate} \underset{PLP}{\overset{ALT}{\rightleftharpoons}} \text{Pyruvate} + \text{L-glutamate}$$

- Asparate Transaminase (AST) / glutamate oxaloacetate transaminase (GOT)
↳

$$\text{L-Aspartate} + \alpha\text{-Ketoglutarate} \underset{PLP}{\overset{AST}{\rightleftharpoons}} \text{Oxaloacetate} + \text{L-glutamate}$$

Most AA Undergoes Transamination Rxn except Lysine, Threonine, Proline, Hydroxyproline

Removal of $-NH_2$ group

(114)

Date
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DEAMINATION

→ It may be oxidative or Non-oxidative;

Oxidative deamination :→

$$\text{L-glutamate} \xrightarrow[\text{L-GDH}]{NAD^+ (\text{or } NADP^+) \;\rightarrow\; NADH (\text{or } NADPH) + H^+} \alpha\text{-Ketoglutarate} + NH_3$$

ATP, GTP, NADH ⊖ → L-GDH ← ⊕ ADP, GDP

- Occurs Primarily in Liver QQ & Kidney.
- GDH (Glutamate dehydrogenase) → Mitochondrial matrix enzyme;
  - Unusual enzyme in being able to utilize both NAD^+ & $NADP^+$ as co-substrates QQQ

NEET-16 Requires Riboflavin in the form of FMN.

Nonoxidative deamination :→ (A) By Amino acid oxidases;

(B) By Amino acid dehydratase;

→ **Transport of Ammonia** :→ (A) In the form of glutamine :→

- In Many tissues like **Liver, kidney & Brain**, NH_3 Combines c̄ glutamate to yield glutamine, by the action of glutamine synthase.

$$\text{Glutamate} + NH_4 \xrightarrow[\text{Glutamine synthetase}]{ATP \;\rightarrow\; ADP + P_i,\; Mg^{2+}} \text{Glutamine}$$

The Brain is Rich Source of glutamine synthase & it predominantly detoxifies NH_3 by this Route.

Teacher's Signature ....

Glutamine is a Non-toxic Major transport form of NH_3.
The glutamine is transported by blood to liver; where deamination (Removal of amino group) of glutamine takes place

$$\text{Glutamine} \xrightarrow[\text{Glutaminase}]{H_2O} \text{Glutamate} + NH_4$$

Renal tubular cells → Maintain Acid base balance by formation & secretion of NH_3.

Metabolic acidosis → Excretion of NH_3 ↑es
Metabolic Alkalosis → Excretion of NH_3 ↓es

(B) In the form of Alanine →

Alanine transports NH_3 from Muscles to liver through "Glucose – Alanine cycle".

Teacher's Signature ....

GENETIC CODE

- It is the system of Nucleotide Sequences of mRNA that determines the sequence of Amino Acids in protein.

- codon : is a sequence of three adjacent bases that corresponds to one amino acid.

- There are 64 codon sequences ; b/c of four Nucleotide bases A, G, C & U. (Thymine is not Involved in codon)

- Characteristics of genetic codes → (A) Universal →
  Each codon specifically codes for Same AA in all species.
  eg → UCA → Serine; CCA → Proline → In all organisms

  Exception → In Human Mitochondria →
  - "UGA" codes for tryptophan instead of Stop codon;
  - "AUA" codes for Methionine instead of Isoleucine;
  - "AGA" & "AGG" serve as stop codon instead of Arginine.
  - "UGA" codes for Selenocysteine by a Mechanism called "Translational recoding".

(B) Unambiguous / specific → A Particular codon always code for the Same AA

(C) Degeneracy / Redundancy → A given AA may have more than one codon;

(D) Stop or termination or Nonsense codon →
UAA → Amber; UAG → Ochre; UGA → opal

(E) Non overlapping & Nonpunctate (Comma less) →
Written as AUGCUAUUGAC

Teacher's Signature ....

Wobble Hypothesis → Degeneracy of codes can be explained by "wobble hypothesis for codon - Anticodon interaction".

- each codon base pairs with "Anticodon of tRNA" in Antiparallel fashion.

- 1st two bases of codon are the same; whereas the third is different, "wobble".

↓

- 1st two bases of codon pairs c̄ last two bases of Anticodon with normal watson - crick base pairing.

↓

- Base Pairing of 3rd base of codon (at 3' end) with 1st base of Anticodon (at 5' end at tRNA) doesn't strictly follow watson - crick base pairing Rule.

↓

This allows a single tRNA to recognize more than one codon

↓

So; No need of 61 tRNA species to Read 61 codons that code for AA.

- Minm of 31 tRNAs are Required to translate all 61 different codons for the amino acids.

Teacher's Signature ....

✓✓✓ TECHNIQUE		SAMPLE ANALYZED	GEL USED	PROBE
1. Southern blot	→	DNA Q	Yes	Radioactive DN.
2. Allele specific oligoNucleotide (ASO)	→	DNA	No	Allele specific oligonucleotide.
3. Microarray	→	m-RNA or C-DNA	No	DNA Probe
4. Northern blot	→	RNA Q	Yes	DNA Probe
5. western (immuno) blot	→	Protein Q	Yes	Labeled Antibody Q
6. South western blot	→	Protein DNA	No	DNA Probe
7. ELISA	→	Proteins or Antibodies	No	Antibody Q (specific for protein to be Measure)
8. Proteomics	→	Protein QQ	Yes	—

Teacher's Signature ....

PYQ — Heme lies in the hydrophobic Interior (pocket) of hemoglobin & Myoglobin Both.

STRUCTURE OF HEMOGLOBIN

Hemoglobin = Globin (contain 4 Polypeptide chains) + 4 Heme

HEME (Most important Type III Porphyrin)
Series IX Porphyrin in Fischer series. (NEET 16)

- derivative of Porphyrin (Protoporphyrin)

composed by fusion of four Pyrrole Ring linked by Methenyl (=CH) bridges i.e tetra-pyrrole Ring.

- Iron is held in the center of Porphyrin Ring in ferrous form (Fe^{2+}).

- Iron has six co-ordinated bonds &

(A) Four bonds b/w the iron & Nitrogen atoms of the Porphyrin Ring System;

(B) Fifth bond is b/w N_2 atoms of histidine residue of globin chain, k/as "Proximal histidine (His F_8)";

(C) Sixth bond is formed c̄ Oxygen.

N.B. → Oxygenated form of Hb is stabilized by the H-bond b/w oxygen & side chain of Another histidine residue of globin chain, k/as "distal histidine (His E_7)."

→ Distal histidine (His E_7) of globin protein creates a hindered environment which drastically reduces affinity of heme for carbon Monoxide (CO).

Teacher's Signature

GLOBIN → Made up of 4 Subunits i-e two pairs of identical subunits.

HbA → $\alpha_2 \beta_2$

HbF → $\alpha_2 \gamma_2$

HbA_2 (Minor hemoglobin) → $\alpha_2 \delta_2$

→ Four Polypeptides Subunits of Hb are held together by Salt bonds, H-bonds, & vander wall forces.

N.B. → The Heme part of Hb is Same in all types of Hb. The protein part (globin) varies in different Hb.

Oxygenation of Hb & co-operativity :-

- Each Molecule of Hb can combine with upto four molecules of oxygen. Q.Q

V.V.Q. 1st O_2 Molecule → binds c̄ greatest difficulty

⇓

↑es affinity to Next O_2 molecules

⇓

4^{th} O_2 Molecule to bind c̄ greatest ease.

⇓

co-operative binding or co-operativity

↳ Responsible for sigmoid shape of the O_2-Hb dissociation curve

- T-form (Tense or Taut or stressed form) is low affinity form; whereas R-form is more oxygen affinity form of Hb.

Teacher's Signature ....

Q8) Conjugation process converts water insoluble (hydrophobic) unconjugated bilirubin into water soluble (hydrophilic) conjugated bilirubin.

HEME DEGRADATION

HEME

↓ Heme oxygenase ($\to CO$, $\to Fe^{+2}$)

Biliverdin

↓

Bilirubin (Unconjugated)

↓ bound to S. albumin

Bilirubin – albumin complex

↓ uptake by liver & did conjugation

Conjugated bilirubin (Bilirubin-diglucuronide)

↓ secretion into bile

*→ Bilirubin is conjugated c̄ glucuronic acid by enzyme UDP-glucuronyl transferase; to form "bilirubin diglucuronide".

after transfer of bilirubin to hepatocytes; it is bound to "Ligandin".

Bilirubin-diglucuronide in intestine

↓

Urobilinogen

80% ↓ — 20% Absorption → Enterohepatic circulation

Stercobilinogen → Urobilinogen (in kidney)

↓ ↓

Stercobilin* (gives brown colour to stool) → Urobilin

↓ ↓

excretion in feces → Excretion in urine

Teacher's Signature ....

INTEGRATION OF METABOLISM

- FED STATE (ABSORPTIVE STATE) → Period (2-4hr) following a meal & characterized by high levels of Nutrients in blood.

→ High Insulin : Glucagon Ratio QQ

- Glucose-6-phosphate has 3 fates in fed state

Utilized in glycolysis	Synthesis of glycogen	Utilized in HMP Shunt.

- In this stage, glucose is the Major Source of energy sparing other (eg → fatty acids) to be stored as energy Reserve.

effect of insulin on Major Metabolic Pathway :-

Carbohydrate Metabolism	effect	Regulated enzyme
• Glycolysis →	↑ →	↑ PFK-I, ↑ Pyruvate Kinase, ↑ Pyruvate Dehydrogenase
Gluconeogenesis →	↓ →	↓ Pyruvate carboxylase, ↓ glucose-6-phosphatase, ↓ PEP carboxykinase
• Glycogenesis →	↑ →	↑ glucokinase QQ, ↑ glycogen synthase
Glycogenolysis →	↓ →	↓ glycogen Phosphorylase

Lipid Metabolism	effect	Regulated enzyme
• Lipogenesis →	↑ →	↑ Acetyl CoA carboxylase; ↑ FA synthase
• Lipolysis →	↓ →	↓ Hormone Sensitive lipase
• Cholesterol synthesis →	↑ →	↑ HMG-CoA Reductase
• TAG Synthesis →	↑ →	↑ glycerol kinase, ↑ Acyl-CoA-glycerol-3P-transferase
• Lipoprotein degradation →	↑ →	↑ Lipoprotein lipase.

Protein Metabolism → effect → Regulated Enzyme

- Protein Synthesis → ↑ → ↑ RNA Polymerase & Ribosome assembly.

- FASTING & STARVATION → Fasting → No food is ingested after the absorptive period (24hr); Starvation → Prolonged severe deprivation of food.
  - ∴ High Glucagon : Insulin Ratio

BODY STORES OF ENERGY

- Largest Reserve of energy in the body → Fat in Adipose tissue. QQ

- 1st Main Provider of energy → Liver glycogen. QQ
  ⇓
  It Maintains the blood glucose levels b/w Meals.

- (1) Brain → No stored fuel in Brain; but it utilized 60% of total energy under Resting conditions. QQ

  - Glucose → Sole fuel of Brain
  - Ketone bodies → ← In Prolonged starvation.

  - Fatty acids don't serve as fuel for the brain
    ⇓
    bloc they are bound to albumin in plasma, so, can't cross blood-brain barrier.

(2) Skeletal Muscles → Major fuels for Muscle are glucose[Q], glycogen[Q], fatty acids[Q] & Ketone bodies[Q].
- After Meal → glucose
- Resting Muscle during Starvation[Q] → FFA[Q]
- During Prolonged Starvation[Q] → Ketone bodies[Q]

During Muscular activity

Immediate energy System	Anaerobic glycolytic System	Aerobic (oxidative) System
Energy production is immediate (0-30 sec);	Energy production is fast (30-180 sec).	Energy Production is slow (>3 min)
Substrate → ATP, creatine phosphate	Glucose or glycogen	Glucose or glycogen, FFA
eg → weight lifting; Short sprints (100m) & jumping.	Longer sprints (200m) & 100m Swim	Jogging & Marathon Run

(3) Heart → FFA Serves as major fuel (60-90%)[QQ]. Ketone bodies, lactate & Some glucose can also serve as the fuel for heart.

(4) Adipose tissue → After Meal → Glucose
During Fasting → Fatty acids
During Prolonged Starvation → Ketone bodies

Teacher's Signature ....

(5) RBCs :→ only glucose is the sole fuel for RBCs. (AI'13)

(6) Liver :→ α-Keto acids derived from degradation of Amino acids are the fuel of liver.

Metabolic alteration during Fasting - Starvation

divided into three categories →

(A) Initial Stage (Lasts upto 2-3 days) :→

*** Liver glycogen Provides energy upto 16-18hrs of Starvation.

Gluconeogenesis ;
Lipolysis ↑ ;
Ketogenesis ↑ ;
Protein degradation ↑ ;
Glycogenolysis .

(B) Intermediate stage (3-24 days) :→

Enhanced these Pathways {
Gluconeogenesis ↑↑
Lipolysis ↑↑
Ketogenesis ↑↑
Ketone body oxidation ↑↑

Slowed these Pathways {
Protein degradation
Protein synthesis

(C) Last Stage (>24 day) :→
Enhanced these Pathways ← {
Ketone body oxidation by brain ↑↑↑
Fatty acid utilization ↑↑↑
Gluconeogenesis ↑↑↑

- During Starvation, Muscle proteins are degraded to serve Amino acids (especially Alanine) as substrate for gluconeogenesis.

MUTATIONS

→ Permanent alteration in genetic material i.e in DNA sequence

Point Mutations (Base Substitution Mutation) | Frameshift Mutations | Trinucleotide repeat Mutation.

⇓

- Substitution of a Single base by another is k/as "Point Mutation".

if Purine replaced by Purine ⇒ TRANSITION
Pyrimidine replaced by Pyrimidine ⇒

if Purine replaced by Pyrimidine ⇒ TRANSVERSION
Pyrimidine replaced by Purine ⇒

→ Silent Mutation → (GGC → GGG) → Both code for glycine.
↳ No change in expression of Protein AA

→ Mis-Sense Mutation → codon containing the changed base codes for different Amino Acids.

- Acceptable mis-sense: eg → "Hb Milwaukee" has glutamic acid in place of valine. "Hb Bristol" has aspartic acid
- Partially acceptable Mis-sense: eg → substitution of glutamate by valine at 6th position of β-chain
- Unacceptable mis-sense: eg → Methemoglobin histidine replaced by tyrosine at 58th position α-chain

→ Non-sense Mutation → Sense codon into Non-sense codon.
↳ Protein Synthesis stops permanently.

Teacher's Signature ......

Frame-Shift Mutation → due to insertion or deletion of one or two bases or three base pairs
⇓ ⇓
Change whole reading frame distal to Mutation | Single AA is Incorporated or deleted, Rest AA has Same sequence

TriNucleotide Repeat Mutation → A codon (i.e trinucleotide sequence) undergoes amplifications & the same codon is repeated so many times.

eg → Huntington's disease (CAG repeat);
Spinocerebellar ataxia (");
Fragile-X Syndrome (GGG or GCC repeat);

Some other types of Mutation →

(A) Splice site mutation → eg → Myotonic dystrophy gene;

(B) Conserved Mutation → Substituted AA has same properties of original AA. Thus; the function of protein is not altered.
eg → glutamic acid replaced by aspartic acid;
Alanine replaced by leucine

(C) Null Mutation (Loss of function Mutation);

(D) Neomorphic Mutation (Gain of function Mutation).

Teacher's Signature ....

Metaphase arrest of the chromosome is Needed. (121)
Pr Probe tagged c̄ Flourscence isothiocyanate is Needed

Date
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FISH (FLUORESCENCE IN SITU HYBRIDIZATION)

- Cytogenetic technique that can be used to detect the presence or absense of specific DNA sequences (specific gene locus) QQ.
- Modified version of DNA-DNA hybridization.
- Can be used in → Rapid identification of chromosome during interphase. QQQ

colchicine
↳ arrest in Metaphase

Used in metaphase cells to detect specific microdeletions.

- Diagnostic → Monitoring the Success of bone marrow transplantation; QQ

Research → Gene Mapping; QQ

Study of 3D chromosome organization in interphase Nuclei. QQ

ONE-LINER QUESTIONS

- Enzyme "Alglucerase" is used in T/t of → "Gaucher's disease"
- UV-visible spectroscopy (UV-vis) can distinguish b/w enantiomers by showing a distinct cotton effect for each isomer.

two Method

~~Octant Rule~~ ~~exciton chirality Method~~

Octant rule   exciton chiralry method

- Method of study of oncogenes ⇒ Transfection. QQ

Teacher's Signature ....

- "Spectrophotometer" ⇒ Measures the amount of light that a Sample absorbs.
  ↳ amount of DNA/RNA can be quantified.

- Comparative genomic hybridization ⇒ used to detect Chromosomal differences b/w Neoplastic cells & their Normal counterparts.

- Starting material for production of Insulin from bacteria
  ⇓
  mRNA from β Pancreatic cells of human.

- Immunohistochemistry ⇒ Used in diagnosis of abnormal Cells such as those found in tumors.
  ↳ also used to understand the distribution & localization of biomarkers

- Gene of Interest is usually administered via either of two vectors :-
  ① Viral vectors → Retrovirus, Adenovirus, Lentivirus, Adeno-associated virus;
  ② Non-viral plasmids; Liposome (cationic - Lipid) complex.
  ⇓
  M/c used vector
  Most powerful cloning vector ⇒ Cosmid.

- Site-directed Mutagenesis ⇒ K/as "oligonucleotide-directed Mutagenesis"
  ⇓
  . Can introduce alteration of selected Regions of DNA molecule i.e directed to specific site.

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- It may involve single base change (point mutation), multiple base changes, deletion or insertion of selected DNA sequence.

- Chromosome walking ⇒ A fragment representing one end of a long piece of DNA is used to isolate another
⇓
It means mutant gene is isolated by using the adjacent gene.
⇓
To locate the mutant gene walking starts at the closest gene

NEET'16

Human Genomic Sequences

- Type I or gene sequences (30%)
  - Exons (1.5%)
  - Introns (98.5%)
- Type II or Transposons or Jumping genes or Moderately Repetitive sequences (45%)
  - Lines
  - Sines
- Miscellaneous sequences (25%)
  - Satellite DNA or Highly Repetitive sequences or Simple segment Repeats (3%)

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LipoArabinomannan (LAM) Antigen in urine ; Seen in Mycobacterium tuberculosis
⇓
It is a lipopolysaccharide present in Mycobacterial cell wall.

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LIPID STORAGE DISEASE

	Disorder	Enzyme defect
1.	Gaucher's disease →	β glucosidase
NEET'16 2.	Niemann Pick disease →	Sphingomyelinase
3.	Fabry's disease →	α galactosidase
4.	GM1 Gangliosidosis →	β galactosidase
NEET'16 5.	GM2 Gangliosidosis / Tay Sach's disease →	Hexosaminidase A
6	Sandhoff's disease →	Hexosaminidase A & B
7	Krabbe's disease →	Galactosyl ceramidase
8.	Metachromatic leukodystrophy →	Aryl sulfatase A
9.	Farber's disease →	Ceramidase
10.	Wolman's disease →	Acid Lipase

* D/O Related to Heme Synthesis :⇒

Features	Dubin Johnson Syndrome	Rotor Syndrome
Liver Appearance →	Black Pigmentation (d/t accumulation of epinephrine)	Normal histology & Appearance
Gall Bladder visualisation on oral cholecystogram →	can't be visualised	can be visualised

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Features	Dubin Johnson Syndrome	Rotor Syndrome
Total coproporphyrin →	Normal but Coproporphyrin-I is more than 80% of total coproporphyrin (Normal is coproporphyrin I < 25% of total)	Elevated but coproporphyrin I is less than 70% of total coproporphyrin.

* One Liners Related to Lipid Metabolism :⇒

- Max^m Lipid content overall ⇒ Chylomicrons
- Min^m Lipid content overall ⇒ HDL

- Max^m Triglyceride content overall ⇒ Chylomicrons
- Max^m exogenous (Dietary) Triglyceride content overall ⇒ Chylomicrons
- Max^m endogenous triglyceride content ⇒ VLDL
- Transport of endogenous triglyceride ⇒ VLDL
- Minimum Triglyceride content ⇒ HDL

- Minimum Cholesterol content ⇒ Chylomicrons
- Maximum Cholesterol content ⇒ LDL

- Maximum phospholipid content ⇒ HDL.
- Minimum phospholipid content ⇒ Chylomicrons

- Lipoprotein c̄ lowest density ⇒ Chylomicrons
- Lipo protein c̄ Max^m density ⇒ HDL
- Lipoprotein c̄ Max^m (largest) size ⇒ Chylomicrons
- Lipoprotein c̄ Smallest size ⇒ HDL

- Lipoprotein c̄ Minimum Protein content ⇒ Chylomicron
- Lipoprotein c̄ Maximum Protein content ⇒ HDL (HDL_2).

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Valine; Isoleucine

Propionyl CO-A (3C)

↓ Propionyl COA Carboxylase ⇢ Propionic aciduria

D-Methyl Malonyl CO-A

↓ Racemase

L-Methyl Malonyl CoA ⇢ Methyl Malonic Aciduria (def. of Mutase enzyme) → Urine

↓ Mutase/Isomerase (B_{12})

Succinyl COA (4C)

↓

TCA

* Receptor	Distribution of Receptor	Apoprotein Identified
LDL Receptor (Clathrin coating)	Hepatic & Extra-hepatic cells	Apo-B-100 as well as Apo E
LRP (LDL Receptor Related protein)	Hepatic	Apo E
Remnant Receptor	Hepatic	Apo E

* Apo D & Apo J (special type of Apoprotein) seen a/w Neuro-degenerative D/O (Alzeimer disease).

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* Antioxident Defense Mechanism

Preventive Measures

i) Efficiency of e- transport chain;

ii) Transition Metal sequestration

iii) Glutathion peroxidase

iv) Catalase

Interceptive Measure

- SOD ⇒ Mitochondria (Mn^{+2})
  - Cytosol (Cu^{+2})
  - extracellular (Cu^{+2} & Zn^{+2})
- Non-enzymatic Substances ⇒
  - Vit. E
  - Vit. C
  - β-carotene
- Others ⇒
  - Caffeine
  - ceruloplasmin
  - Vit. A
  - Cysteine

* Iron containing Protein

Heme containing :- Hb / Mb
Cytochrome
Tryptophan pyrrolase
Catalase
Peroxidas
NOS (Nitric oxide Synthase)

Fe-S complex :- complex II
SOD
Xanthine oxidase.

Non-heme containing :- Phenylalanine hydroxylase
Aconitase
Ferritin
Transferrin

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Enzyme Regulated by Ca^{+2}

- Adenyl cyclase
- Ca-Mg ATPase
- G3P dehydrogenase
- Glycogen synthase
- Phospholipase C
- Pyruvate carboxylase
- PDH
- Pyruvate kinase

* Mineral — Enzyme

Mg ⟶ Hexokinase, enolase; PFK; Glu-6-phosphatase; DNA Polymerase

Mn ⟶ Mn as a part of enzyme→ Arginase; Pyruvate carboxylase; Mn SOD

Mn as an activator :→
- Glucosyl transferase, PEP Carboxykinase; Glutamine Synthetase
- Hydrolase, Kinase; Decarboxylase, Transferase

Mo ⟶ Xanthine oxidase
Aldehyde oxidase
Sulfite oxidase

Se ⟶ Glutathione Peroxidase; Iodo-thyronine deiodinase

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* Sanger's Reagent (1-Fluoro, 2-4-Dinitrobenzene) ⇒ Used to determine AA Sequence.

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* Reactions or Test to Identify specific Groups or Amino groups

:⇒ Reactions — specific group or Amino Acid

* Biuret Rx^n ⟶ Two peptide Linkages.
* Ninhydrin Rx^n ⟶ α- AA
* Xanthoproteic Rx^n ⟶ Benzene Ring of Aromatic Amino Acids.
* Millon's Rx^n ⟶ Phenolic group (Tyrosine)
* Hopkins - cole Rx^n ⟶ Indole Ring (Tryptophan)
* Sakaguchi Rx^n ⟶ Guanidine group (Arginine)
* Nitroprusside Rx^n ⟶ Sulfhydryl groups (cysteine)
* Sulfur test ⟶ Sulfhydryl group (cysteine)
* Pauly's test ⟶ Imidazole Ring (Histidine)
* Folin Ciocalteau's test ⟶ Phenolic group (Tyrosine).

* Properties of An Amino acids @ Isoelectric pH are :⇒
  - Maximum precipitability;
  - Minimum buffering action (Max^m buffering action is when the pH = pK & Not @ Isoelectric pH
  - Minimum Solubility
  - No Mobility in an electric field.

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* **Amino Acids & their Biological Amines :⇒**

Amino Acids		Biological Amines
• Histidine	⟶	Histamine
• Tyrosine	⟶	Tyramine
• Tryptophan	⟶	Tryptamine
• Lysine	⟶	cadaverine
• Glutamic Acid	⟶	GABA
• Serine	⟶	Ethanolamine
• Cysteine	⟶	β-Mercapto Ethanolamine

* **CATABOLISM OF GLYCINE**

- By Glycine clevage system :⇒ consists of three enzyme & an " H-protein".

↳ Glycine dehydrogenase
Amino Methyl-transferase
Dihydrolipoamide dehydrogenase

* **Use of Recombinant DNA technology**

A. In Medicine → Insulin; growth hormone; TNF; IL-2; Prourokinase; Taxol; Interferon; DNA vaccine; Hepatitis B vaccine; Antibiotics; Monoclonal Ab

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B. Agriculture ⇒ Crop $\bar{c}$ Insectiside Resistance; delayed Ripening; Nutritious.

C. Animal Husbandry ⇒ More Milk production
DOLLY :- Transgenic clone.

D. Gene Library / DNA Library.

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